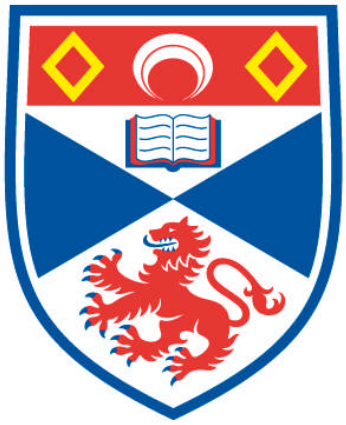
PHENOTYPIC PLASTICITY IN THERMAL TOLERANCE: LIFE HISTORY STRATEGY OF AN INVASIVE FRESHWATER FISH

Al J. Reeve

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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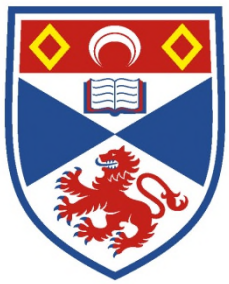

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Themes


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Abstract

Background

Temperature has a fundamental effect on organisms because it alters the speed of biochemical reactions and thus metabolism. This influence scales up to have ecosystem wide effects as the life history strategies of individual species differ in response to temperature. With the prospect of increasing global temperatures ecosystem functions could be interrupted. In order to predict the consequences of changing environmental conditions it is important to first establish how fitness related traits are affected by changing thermal conditions.

Aims

The aim of this thesis was to develop a detailed understanding of the thermal niche of an invasive, tropical freshwater fish species.

Methods

Using ecologically realistic conditions this thesis investigates the effect of environmental variation within and between generations on behavioural, growth, physiological, and reproductive characteristics of the Trinidadian guppy (*Poecilia reticulata*).

Results

The results provide an insight into the fundamental thermal niche of a widely used model fish species as well as detailed measures of how thermal change alters phenotypic characteristics. Guppies are demonstrated to have a broad thermal tolerance and be phenotypically responsive to changing environmental conditions. The results also suggest that environmental characteristics of the guppy's habitat make an important contribution to the differences observed between populations of guppies in Trinidad.

Conclusions

Water temperature in the guppy's natural environment varies widely over a daily cycle and I suggest that this is partly responsible for the guppy becoming phenotypically plastic and thermally tolerant. Furthermore, phenotypic flexibility is an important characteristic that will enable guppies to withstand some climate warming and continue to expand their invasive range poleward.

Using experimental conditions which resemble those in the natural environment is important for developing accurate model parameters. These are necessary for predicting the ecosystem effects of environmental variation and for adaptive mitigation or pre-emptive management of range extensions by invasive species.

Acknowledgements

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Alfredo, for broadening my horizons and always being ready with paper and pen to draw an explanation, and

Anne, for insightful comment and critique and always making me focus on the question.

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My PhD journey was made one million times more enjoyable by Clara, Dani, Chad, Alicia, Helen, Tom, Grant, Mark, Rashmi, Donald, Mora, Silje, Joe, Laura and the Whip Inn Boys (past and present);

And, of course, everything is made one million times better with Meriem and Stanley by my side.

I would like to thank my parents and all of these people for supporting me every step of the way.

Thanks!

Xxx

Dedication

For Cathie Wilson, Christine Cartwright & Stanley Kayoueche Reeve

I've met some people along the way,
Some of them split some of them stay,
Some of them walk some walk on by,
I've got a few friends I'll love till I die
From all of these people I try to learn,
Some of them shine some of them burn,
Some of them rise some of them fall,
For good or bad I've known them all

We live our life in our own way,
Never really listened to what they say,
The kind of faith that doesn't fade away
We are the true believers
We are the true believers

Well you can fight or you can run,
Hide under a rock till the war is won,
Play it safe and don't make a sound,
But not us we won't back down
True believers all the way,
You and I...

Attonito : Steinkopf : Kienlen : McDermott

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Acronyms

BAH: Beneficial Acclimation Hypothesis

HIREC: Human Induced Rapid Environmental Change

ROS: Reactive Oxygen Species

sd: Standard Deviation

se: Standard error

SGR: Specific Growth Rate (SGR)

sl: Standard Length

TPC: Thermal Performance Curve

$\mathbf{T_{opt}}$**:** Thermal optimum (temperature were performance is maximised)

$\mathbf{P_{max}}$**:** Performance maximum (highest performance across all temperatures)

$\mathbf{CT_{min}}$**:** Critical thermal minimum (upper temperature where performance is zero)

$\mathbf{CT_{max}}$**:** Critical thermal maximum (upper temperature where performance is zero)

$\mathbf{T_b}$**:** Body temperature

$\mathbf{T_O}$**:** Optimal body temperature

$_{\mathbf{r\ max}}$**:** Temperature at which 'fitness' is maximised

Glossary

Acclimation: Any facultative modification in a physiological trait in response to changes in an environmental variable in the lab. Changes can be in response to the developmental environment or long-term environmental shifts during the later stages of the life history of an organism (Wilson and Franklin 2002).

Beneficial Acclimation Hypothesis: proposes that all acclimation changes enhance the physiological performance or fitness of an individual organism (Wilson and Franklin 2002).

Bigger is better: Proposes that individuals with larger body size will tend to have greater performance and fitness than smaller individuals within a population (Kingsolver and Huey 2008).

Developmental plasticity (developmental effects): phenotypic changes induced by variation in the developmental environment. Changes can include facultative responses by the organism (e.g. acclimation) or unavoidable biophysical consequences of the environment (obligatory and/or pathological) (Wilson and Franklin 2002).

Fundamental thermal niche: the range of temperatures over which a species continues to operate in laboratory environment (Magnuson et al. 1979).

Hotter is better: Proposes that genotypes or species with relatively high optimal temperatures also have relatively high maximal performance or fitness (Kingsolver and Huey 2008).

Hotter is smaller (temperature-size rule): The temperature–size rule proposes that ectotherms that develop at higher temperatures will be relatively small as adults – that is, hotter is smaller (Kingsolver and Huey 2008).

Oxidative stress: an imbalance arising when the rate of production of reactive oxygen species (ROS) exceeds the capacity of the antioxidant defence and repair mechanisms, leading to oxidative damage (Metcalfe and Alonso-Alvarez 2010).

Performance: A measure of an organism's capacity to function, usually expressed as a rate or probability (Angilletta 2009).

Phenotypic plasticity (phenotypic flexibility): environmentally induced variation in morphology, physiology and/or behaviour of an organism (Wilson and Franklin 2002).

Realised thermal niche: realized (field) thermal niches tend to be narrower than fundamental (laboratory) niches, in a large part because of species interactions (Magnuson et al. 1979).

Temperature-size rule: see Hotter is smaller.

Thermal generalist: performance is maintained over a wide range of temperatures (Nilsson-Örtman et al. 2012).

Thermal specialist: performance is restricted to a narrow range of temperatures (Nilsson-Örtman et al. 2012).

Prologue

Prologue

Environmental conditions, especially temperature, impact upon all aspects of ectotherm biology (Angilletta 2009); conspire to modulate the diversity of ecosystems (Stevens 1989); and contrive to define macroecological patterns of distribution (Gaston et al. 2009).

Temperature has a fundamental effect on biochemical reactions by altering enzyme efficiency and consequently, metabolic rate is intrinsically linked to temperature (Hochachka and Somero 1984). Ectothermic species are especially sensitive to temperature as they rely on external heat sources to maintain body temperatures within a relatively narrow range (Martin and Huey 2008). The result is that temperatures outside of this optimal range can dramatically impact upon an individual's energy requirements and thus alter resource acquisition and allocation. Consequently, environmental temperature directly impacts upon fitness by altering performance, life history traits, activity patterns and secondary sexual characteristics (Cossins and Bowler 1987). Furthermore, alterations to fitness traits at an individual level can have ecosystem-wide consequences through, for example, the interruption of predator-prey interactions (e.g. Ferris and Wilson 2012, Grigaltchik et al. 2012) or changes in phenology (Bradshaw and Holzapfel 2010, McNamara et al. 2011).

The ability of individuals to respond to variation in temperature (or other factors) depends on the breadth of their phenotypic flexibility (see **Glossary** for definitions). By expressing different phenotypes based on current, or future, environmental conditions individuals attempt to maximise the performance of

fitness traits (Huey et al. 1999) and the more flexible the phenotype the greater the ability to withstand environmental change. Individuals can respond to daily or short term variability in temperature through physiological and behavioural changes and longer or seasonal fluctuations through broader cellular and physiological changes that results in thermal acclimation (Pigliucci 2010). These responses are generally reversible and allow an individual to differentially express phenotypes as conditions alter.

The temperature experienced during early life history, however, can have lifetime consequences by irreversibly fixing phenotypic characteristics such as size, morphology and thermal tolerance (Atkinson 1996). An individual's phenotype can therefore be influenced across generations as maternal (Uller 2008) and grandmaternal (Lock 2012) conditions can affect aspects of life history. The effects of phenotypic plasticity are far reaching temporally, acting across multiple generations, and geographically, by altering ecosystem dynamics (Miner et al. 2005).

The extent of phenotypic plasticity of which an individual is capable develops as an evolutionary response to variability in their environmental conditions (Kassen 2002). Generally, species which evolved in variable thermal conditions have greater phenotypic plasticity and thermal tolerances than species that experienced a narrower range of temperatures (Bozinovic et al. 2011).

An individual's life history characteristics and breadth of phenotypic plasticity, combined with competitive interactions, dictates the thermal niche within which they live (Kingsolver 2009). Individual-level traits also define

ecosystem structure and the geographic extent to which a species inhabits (Calosi et al. 2010). Changing climate conditions which result in temperature increases or decreases and more frequent occurrence of extreme weather events, however, will have unresolved impacts on individuals and consequently whole ecosystems (Sih et al. 2011).

Species that have flexible phenotypes are more likely to respond successfully to changes in thermal conditions by altering aspects of life history to survive in the novel conditions (Ketola et al. 2013). Furthermore, generalist species tend to have accumulated mutations within their genotype on which selective pressure can operate (Moczek et al. 2011). These species that are more capable of withstanding change are also those more likely to expand their range as the climate changes or successfully invade new habitats given the opportunity (Garcia-Berthou 2007).

Increasing temperature causes an exponential increase in biochemical reaction rates (Gillooly et al. 2001). As tropical species are already near the upper limit of their thermal tolerances they are also most at risk to rising global temperatures (Tewksbury et al. 2008). Freshwater ectotherms are especially vulnerable to changing thermal conditions as they are restricted in their dispersal ability by geographic barriers (e.g. De La Vega-Salazar et al. 2003). This restricts the ability of freshwater organisms to move with climatic shifts and places pressure on them to withstand changes *in situ* (Rosenfield 2002). Furthermore, although accounting for around 0.8% of the Earth's surface, global freshwater supports nearly 6% – around 100 000 – of described species, and is already

suffering serious declines in diversity and so represents an important conservation priority (Dudgeon et al. 2006, Strayer and Dudgeon 2010).

In the early stages of environmental variation, as changes in genetic frequencies take generations to occur, plasticity represents a species' immediate response to new conditions. Prior to adaptation, the life history trade-offs and behavioural changes that enable species to withstand thermal variability and the consequences for the ecosystem are unclear. In order to forecast possible repercussions and attempt to mitigate negative effects, understanding how species adjust important components of their life history in response to changing environmental conditions is essential (Araújo et al. 2005, Matthews and Marsh-Matthews 2011).

Introduction

Introduction

In this **introduction** I will discuss the effects of environmental temperature on ectotherm biology and describe how species withstand variation in thermal regime. I will review examples from a variety of taxa showing that ecosystem dynamics are controlled by temperature and would be disrupted by climate change. I will then make the case that fully understanding the fundamental thermal niche of a species will improve the power of models predicting the effects of climate change.

Temperature

Temperature has a pervasive effect across all levels of organisation from molecules to ecosystems (Hochachka and Somero 2002) and a direct effect on an individual's physiology in two principal ways. Firstly, the fluidity of phospholipids comprising cellular membranes is reduced at low temperatures whereas extremely high temperatures cause a loss of membrane integrity (Hazel 1995). Secondly, protein structure is thermally sensitive and different temperatures alter the structure of substrate binding sites and, consequently, the efficiency of enzymes (Hochachka and Somero 2002). As temperature increases, enzyme efficiency, and thus energy and oxygen consumption also increases resulting in a higher metabolic rate.

Thermal dependence at a cellular level has been demonstrated to affect individual level traits (such as behaviour, growth, survival) through to broad ecological patterns such as population density, diversity and geographic distribution (reviewed in Cossins and Bowler 1987, Johnston and Bennett 1996, Angilletta 2009). Outside of the physiological temperature range to which an

individual is adapted, cell function is compromised and results in myriad consequences. Accordingly, almost all activities a species undertakes is dependent upon, or influenced by, its thermal environment (Johnston and Bennett 1996). Changes to environmental temperature, therefore, can have ecosystem-wide effects through impacts on an individual's fitness, survival and reproductive biology (Seebacher and Franklin 2012).

Each population of species is adapted to a range of temperatures where physiological performance is optimal based on their evolutionary thermal experience. Exposure to temperatures outside of this range, cause function and performance decreases until activity and, eventually, survival is compromised and the species is unable to exist **(Figure 1a)**. This range of temperatures coupled with the effect of temperature on performance traits is integral to determining the geographical distribution and preferred habitat conditions of each species (Schulte et al. 2011).

Almost all known species inhabit a thermal range which is a subset of temperatures within 0-40^{0}C although there are some species that are adapted to exist outside of this range. *Thermophiles*, species that inhabit temperatures between 40-120+^{0}C and *psychrophiles*, that inhabit temperatures down to -20^{0}C, are limited predominantly to archaea and bacteria. There are a few representatives from the Animalia kingdom including the polychaete *Alvinella pompejana*, arthropods from the Grylloblattidae family, and tardigrades (phylum tardigrada) which are capable of surviving short exposure to -272^{0}C and 151^{0}C (Horikawa 2012). Other species have developed adaptations to periodic extreme temperature

exposure such as the larvae of western spruce budworm (*Choristoneura freemani*) which produce silken hibernacula to overwinter as temperatures can reach -40^{0}C (Graether and Sykes 2004) while many of the fish from the Nototheniidae family which inhabit sub-zero Antarctic waters produce antifreeze glycoproteins to inhibit ice crystals forming in blood and body fluids in order to extend their temperature tolerance range by a few degrees (for review see Fletcher et al. 2001).

For the overwhelming majority of species, whose normal activity is within the 0-40^{0}C thermal range, the subset of temperatures an individual can tolerate represents their *thermal tolerance range* and is generally a product of their thermal evolutionary history. Broadly speaking, with increasing latitude, maximum temperatures decrease while daily and seasonal variation increases. Consequently species closer to the equator tend to have narrower thermal tolerances than those at lower latitudes (Janzen 1967, Tewksbury et al. 2008, Sunday et al. 2011). This general pattern differs slightly between aquatic and terrestrial organisms as the upper and lower limits of aquatic organisms both decrease with increasing latitudes while only the lower limit tends to decrease for terrestrial species – possibly as a result of inertia effects of water dampening the variability of temperature within aquatic systems (Sunday et al. 2011). This variability and range of temperatures experienced at lower latitudes is hypothesised to result in broader thermal tolerance ranges enabling a greater geographical distribution (Calosi et al. 2010). Species in the tropics, conversely, are predicted to have respectively narrower ranges and has been suggested to be one of the reasons why there is greater species richness in the tropics (Stevens 1989).

Quantifying the effect of temperature

The relationship between temperature and an individual's performance traits (whether whole-organism or physiological) is termed the thermal performance curve (TPC) (Huey and Stevenson 1979). TPCs enable the thermal performance of individuals to be described and allow con- and heterospecific responses to thermal change, or different environments, to be compared. Plotting the value of a fitness trait along a continuous range of temperatures enables the effect of temperature on performance to be described using non-linear regression **(Figure 1a)**. Performance curves typically have an asymmetrical shape whereby performance increases with increasing temperature before reaching an apogee and then decreasing rapidly (Angilletta 2006).

The upper and lower temperatures where performance is zero are the critical thermal maximum (CT_{max}) and minimum (CT_{min}) and describe the thermal tolerance range for a particular trait. The temperature at which trait performance is maximised is the thermal optimum (T_{opt}) and the highest point of the curve is the maximal performance (P_{max}). More arbitrary thresholds can also be used to describe characteristics such as the optimal performance breadth which is the range of temperatures where performance is at least 80% of P_{max} (Angilletta et al. 2002, Schulte et al. 2011). Variability in the shape of the curve provides a measure of thermal plasticity and enables comparison within and between species (Angilletta 2009).

Curves which cover a relatively narrow temperature range are indicative of *thermal specialists* whereas broad curves indicate *thermal generalists* **(Figure 1b)**.

These descriptions are somewhat subjective, however, and depend on the scale at which species are labelled and compared. Regardless, the asymmetric shape of the relationship between performance and temperature means that for species with narrow TPCs the effect on performance will be much greater than for species with broad tolerance.

While the width and height of the curve provides information on the breadth and performance optimum of the trait, the shape of the curve can be described by a mathematical function. Given a broad enough temperature range, which spans both the ascending and descending branches of the TPC, a second or third order polynomial function usually provides the best description.

Phenotypic plasticity alters the position, breadth or height of the thermal performance curve and thus the performance of the fitness trait under investigation. If, after acclimation to a different thermal regime, an individual can maintain similar performance levels (P_{max}) and the T_{opt} is similar to the novel regime they have successfully (and completely) acclimated. A change in T_{opt} with a reduction in P_{max} suggests incomplete acclimation while no change in T_{opt} and a reduction in P_{max} indicates the individual was incapable of acclimating to the new temperature.

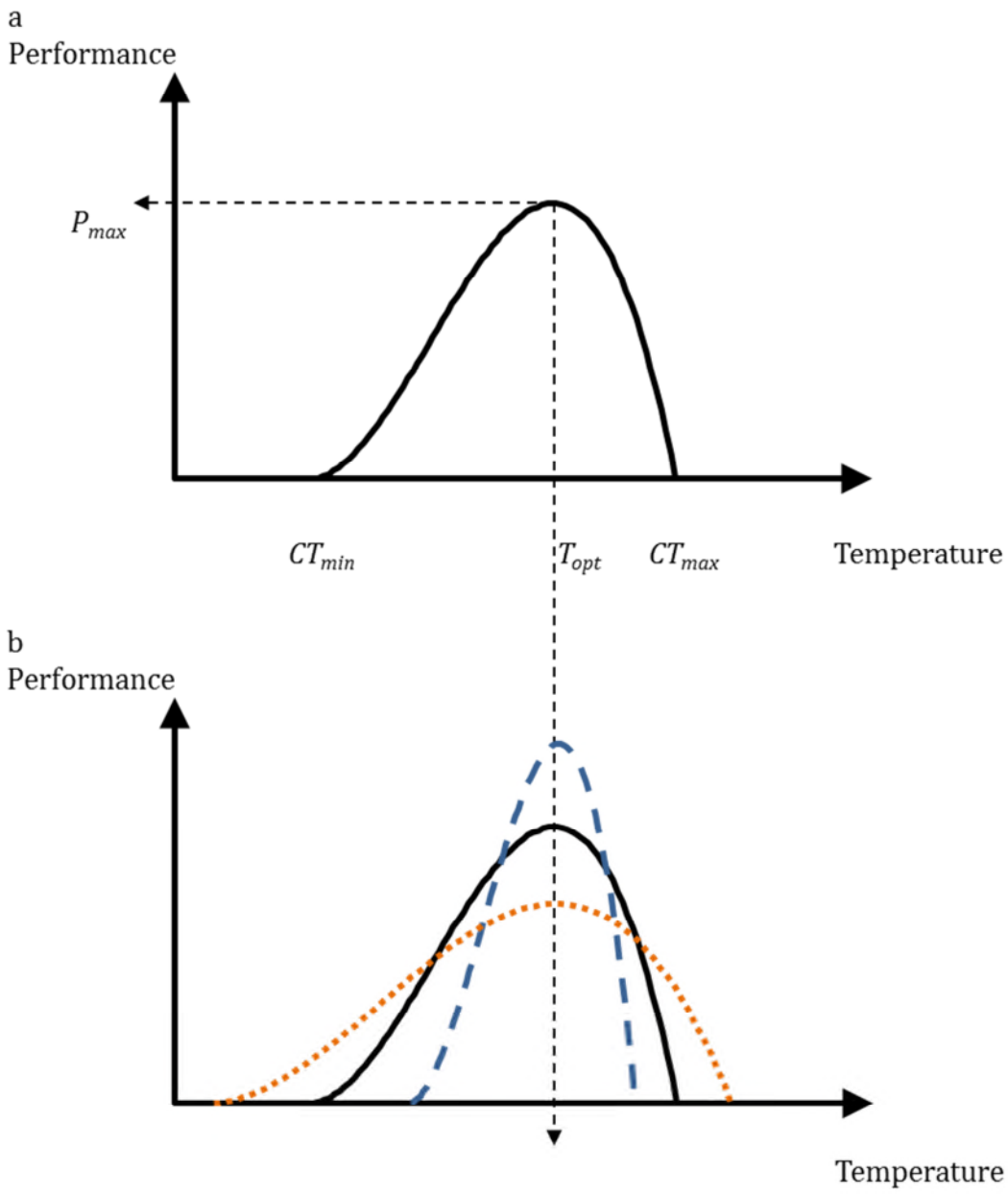


Figure 1: a) Characteristics of a typical thermal performance curve (TPC). As temperature increases, performance also increases until the temperature (T_{opt}) where performance is maximised (P_{max}). Above this temperature, performance declines rapidly until the temperature at which it becomes zero (CT_{max}). **b)** Typical specialist and generalist TPCs centred on the same T_{opt}. Specialists have a narrower thermal range but increased performance compared to generalists.

Oxygen limitation is a relatively recent approach used to describe an organism's thermal performance breadth and explain the asymmetric shape of TPCs (Pörtner 2010). In this theoretical approach, thermal limitation arises because of a mismatch between oxygen supply and demand and results in a loss of

performance and, ultimately, death **(Figure 2)**. The pejus ("getting worse") temperatures (Tp) are the points either side of optimal aerobic performance. Outside of Tp oxygen becomes increasingly more limiting causing hypoxaemia before a critical temperature (Tc) is reached when anaerobic respiration takes over. Finally denaturation temperatures (Td) cause the loss of structural integrity at the molecular level (Pörtner 2010). This model thus describes the link between oxygen supply and performance on an ecosystem scale and oxidative stress has been hypothesised as the ultimate cause of changes in species distribution as a result of environmental temperature change (Pörtner and Knust 2007).

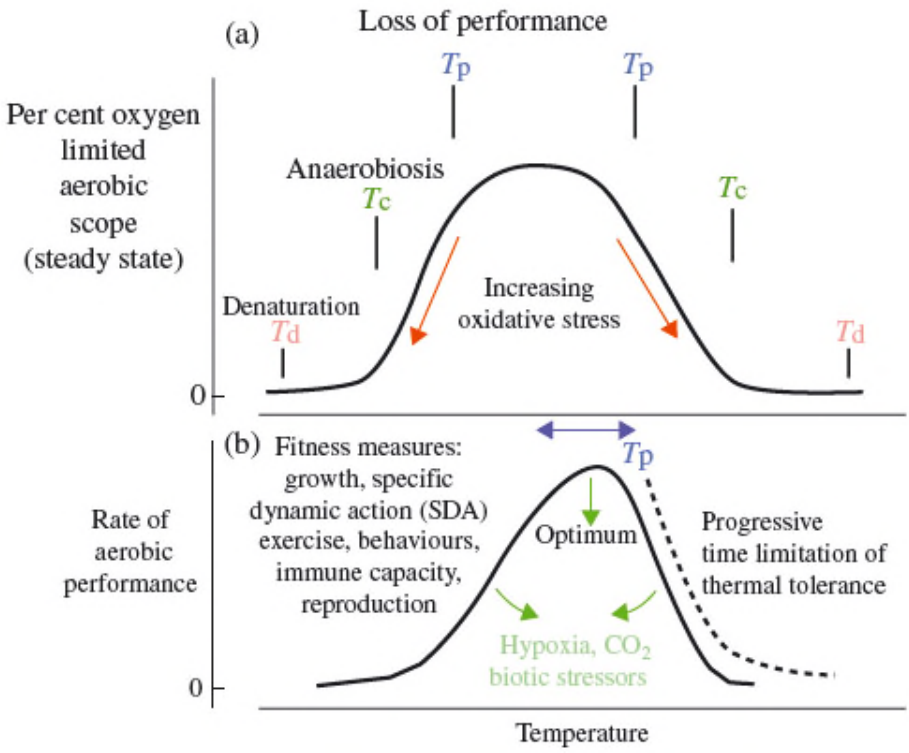


Figure 2: Pörtner's oxygen limitation model for explaining species performance and distribution. At elevated and lowered temperatures oxygen supply becomes limiting. This results in the asymmetric performance curve and limits the thermal niche of an organism. Modified from Pörtner and Peck (2010).

Phenotypic plasticity

Most species face cyclical changes in temperature, whether over a daily or seasonal cycle, and have mechanisms to respond to such predictable changes (Whitman and Agrawal 2008). When faced with variation in the environment, phenotypic plasticity enables an individual to express different phenotypes in response to the prevailing environmental conditions (Pigliucci 2010). Differential phenotypic expression allows individuals to maximise performance traits according to the environment and is, generally, reversible as conditions change (Wilson and Franklin 2002).

Ectotherms are particularly sensitive to temperature as they are dependent on environmental heat sources to maintain body temperature within an optimal range (Martin and Huey 2008). Sudden changes in temperature have immediate effects on ionic equilibrium and acid-base balance (Crawshaw et al. 1982). Behavioural responses are important when responding to these events (see below) however cellular level phenotypic changes can also take place within minutes through the response of important molecules such as heat shock proteins (Fangue et al. 2009, Fangue et al. 2011). In response to daily variability in temperature, ectotherms can perform behavioural or temporal thermoregulation in order to maintain the body at the temperatures which optimise metabolic processes (e.g. Kiefer et al. 2006, Bertolo et al. 2011) or to avoid detrimental conditions (e.g. Biro 1998, Vidal et al. 2011).

Furthermore the body temperature which maximises the fitness of activities such as foraging and digestion may differ. Juvenile sculpin (*Cottus*

extensus), for example, increase their digestion rate by undertaking vertical migrations to warmer surface waters at night. This enables a shorter gastric emptying time, allows for a greater volume of food to be consumed and ultimately results in faster growth (Neverman and Wurtsbaugh 1994). Other activities affected by thermoregulatory behaviour include locomotor performance (e.g. Besson and Cree 2010), metabolic rate (e.g. Speed et al. 2012) and reproductive phenology (e.g. Lacey et al. 2012).

Organisms can respond to (or anticipate) seasonal fluctuations of environmental temperature or prolonged exposure to a novel temperature by changing physiological traits through *thermal acclimation*. Acclimation causes a shift in thermal performance curves through changes in protein expression (e.g. Reyes et al. 2003, Schulte 2004) and cell membrane structure (e.g. Hazel 1995, Overgaard et al. 2005). Alterations to mitochondrial capacity and other oxygen transport chain components also enable physiological maintenance costs to be met with sufficient oxygen (Pörtner 2010). Consequently, acclimation alters the height and breadth of the thermal window (Beitinger and Bennett 2000), seasonal temperature tolerances (Fangue and Bennett 2003) as well as functional and behavioural locomotory ability (Johnston and Temple 2002) to optimise performance within the novel environment.

For a number of years, the fitness benefits of acclimation were assumed rather than tested (see Huey and Berrigan 1996). The Beneficial Acclimation Hypothesis (BAH) suggested that acclimation responses enhance performance traits in the acclimation environment so that there is no loss of performance from

the previous conditions (Leroi et al. 1994). Support for the BAH, however, is mixed (reviewed in Wilson and Franklin 2002, Angilletta 2009) and depends upon the scale and fitness-variable measured and the conditions experienced throughout the organism's life (Scott and Johnston 2012). There are a number of potential costs associated with acclimation centred on the amount of energy required to acclimate and the speed with which acclimation can take place (Hawkins 1995, Huey and Berrigan 1996). Accordingly, the BAH is probably too simplistic an approach and has been superseded by optimality models which incorporate the costs and benefits of associated acclimation (for examples see Gabriel and Lynch 1992, Angilletta 2009).

Cells undergoing changes during development are particularly sensitive to the thermal environment and consequently *developmental plasticity* can affect many aspects of life history (Atkinson 1996). Whereas the responses to changes in temperature that take place within an organism's lifetime are generally reversible, the temperature experienced during development often has permanent consequences by 'fixing' phenotypes (Wilson and Franklin 2002, Beldade et al. 2011). Developmental switches that determine a range of traits such as wing development (Braendle et al. 2006), colouration (Beldade and Brakefield 2002), sex (Ospina-Álvarez and Piferrer 2008), muscle growth (Garcia de la serrana et al. 2012) and adult thermal tolerance limits (Otto 1974, Schaefer and Ryan 2006) are activated by environmentally induced gene expression, most likely through DNA methylation hormonal effects (reviewed in Beldade et al. 2011).

The environmental conditions an organism experiences during its own lifetime can also have phenotypic effects on its offspring through epigenetic process such as DNA methylation (Richards 2006, Angers et al. 2010). *Transgenerational plasticity* has been demonstrated to alter various life history traits such as offspring size and dispersal ability (Burgess and Marshall 2011), egg size and number (Stearns and Koella 1986), growth (Salinas and Munch 2012) and the choice of annual or biennial life history (Galloway and Etterson 2007). The ability for a parent to ameliorate the effect of changes in predation pressure or environmental conditions for their offspring has important ecological and adaptive consequences (e.g. Mousseau and Fox 1998, Rasanen and Kruuk 2007, Dyer et al. 2010). Where there are predictable or minimal levels of intergenerational environmental heterogeneity, transgenerational plasticity can benefit both parent and offspring (Uller 2008). Parent-offspring conflict arises, however, when transgenerational plasticity has a cost for the parent, offspring or both often with the trade-off often being between offspring size and parental lifetime fecundity (Godfray and Parker 1991). The evolutionary significance of such a trade-off is still under scrutiny (Marshall et al. 2010, Steiger 2013).

The adaptive significance of phenotypic plasticity has been subject to a long history of research (e.g. Via et al. 1995, DeWitt et al. 1998) with debate surrounding whether plasticity facilitates or hinders the evolution of new traits which enable an organism to exist in new environments (Ghalambor et al. 2007). Discussion centres on the idea that plasticity buffers the effect of natural selection and therefore slows the rate of adaptive evolution or, conversely, plasticity allows an organism to persist in novel conditions and direct selection acts on the

environmentally induced traits which are eventually assimilated or accommodated (reviewed in Crispo 2008, Badyaev 2009). There is broad agreement that plasticity plays a positive role in diversification of phenotypes (Pfennig et al. 2010) and that developmental plasticity in particular can result in variation for selective forces to work upon (Beldade et al. 2011, Moczek et al. 2011). Consequently, species that are more phenotypically flexible have an increased likelihood of successfully responding, and eventually adapting to, changing environmental conditions.

Origin and maintenance of phenotypic plasticity

Temperature varies over different time scales: rapid or short term, prolonged (seasonal) and across generations. Fluctuating temperature has been cited as the selective pressure resulting in the evolution of broad thermal tolerance or plasticity (e.g. Lynch and Gabriel 1987, Kassen 2002) and unpredictability in those fluctuations has been further cited as important in evolving a wide thermal niche (Kussell and Leibler 2005, Beaumont et al. 2009). Variation in thermal experience can select for thermal generalism by favouring energetic efficiency (Parsons 2005); accumulating mutations and thus selecting for 'evolvability' (Lee and Gelembiuk 2008); or through specific adaptations such as increased expression of heat shock proteins (Ketola et al. 2004).

The variability in temperature that decreases with increasing latitude is linked with the general occurrence of wider thermal tolerances in species further from the equator (Addo-Bediako et al. 2000, Ghalambor et al. 2006). However, temperature variation also takes place over much smaller geographical – and altitudinal – scales and has resulted in differences in organismal thermal flexibility

within habitats depending on how species have adapted to variability. Consequently species assemblages can be maintained through differences in thermal niche and behavioural thermoregulation capabilities that would otherwise cause organisms to exploit similar habitats and resources (e.g. Ostrand and Wilde 2001).

Optimality models attempting to determine the conditions that lead to selection for specialism or generalism conclude that increased response times to environmental change, a stochastic pattern of environmental variability as well as an incomplete knowledge of the environment are all important for the evolution of generalism (Gabriel et al. 2005). In models specifically investigating the effect of thermal variability, specialism was favoured in constant environments or in environments with high within-generation variability. However when there is high between, but limited within, generation variability thermal generalism is more likely (Gilchrist 1995).

One of the implicit assumptions in discussions and models investigating the evolution of thermal generalism is encapsulated by the proverb 'a jack-of-all-trades is a master of none' (see Palaima 2007 and references therein). The assumption here is that there is a trade-off associated with generalism whereby the ability to perform in a broader range of temperatures comes with the cost of performing at a lower maximal performance (Huey and Hertz 1984, **Figure 1b**).

The specialist-generalist trade-off suggests that generalists can perform over a broad range of temperatures but suffer a cost in each environment they enter. A specialist, conversely, will always outperform a generalist in the

environment for which they are specialised (Huey and Hertz 1984, Gilchrist 1995, Gilchrist 1996). However numerous studies have demonstrated generalists to outperform specialists in shared environmental conditions suggesting that specialist-generalist trade-offs can be avoided (Angilletta 2009, Remold 2012). Instead it is proposed that while some trade-offs are along the specialist-generalist continuum, other trade-offs relating to resource acquisition or allocation can circumvent performance loss (Angilletta et al. 2003). Extra time spent acquiring resources can allow an individual to maintain performance although this increases the probability of being killed or parasitized. Similarly, an individual can differentially allocate resources, where the trade-off is that one function is allocated resources at the expense of another.

Regardless of the mechanism by which performance is maintained, ectotherms generally endeavour to maintain their body temperature (T_b) within a narrow range which presumably maximises performance (Angilletta et al. 2006). Within this range it is further assumed that the temperature at which fitness (r) is maximised ($T_{r\,max}$) will be the optimal body temperature (T_O) (Angilletta 2009). However an optimality model developed by Martin and Huey (2008) indicated that T_O should be slightly lower than $T_{r\,max}$ and was supported by 76.2% of species in a meta-analysis of 63 lizard species. This mismatch between T_O and $T_{r\,max}$ is due, in part, because ectotherms are rarely perfect thermoregulators and because the asymmetric shape of TPCs means that an increase in temperature will cause a greater reduction in performance than the equivalent decrease. Martin and Huey further hypothesise that in fluctuating environmental conditions T_O will be further from $T_{r\,max}$ which will consequently have a more pronounced effect on thermal

specialist species who suffer a proportionally larger decrease in performance than thermal generalists over the same range of temperatures. This idea that 'suboptimal is optimal' has only received limited attention although some studies have supported these predictions (e.g. Ragland and Kingsolver 2008, Foray et al. 2014).

Effect of temperature on biology

Biochemical activity rates, such as those of metabolic pathways, all increase exponentially with increasing temperature. The outcome of this is increases in important life history traits such as locomotor speed, digestion rate and fecundity with concurrent decreases in development time and body size (Johnston and Bennett 1996). This fundamental effect on reaction rates is also cited as being responsible for the broad-scale patterns and gradients of biodiversity (Hawkins et al. 2007). In an attempt to link organismal level effects on metabolism and body size scale up to population and ecosystem scales the *metabolic theory of ecology* was proposed (Brown et al. 2004). Using basic principles of biochemical kinetics and thermodynamics the *metabolic theory of ecology* attempts to explain macroecological patterns of ectotherm diversity (Bailly et al. 2014).

The consequences of faster metabolism, however, are higher energy demands. An increase in energy requirements causes a trade-off in resource allocation as less energy is available to be split between growth, reproduction and maintenance or more is being spent on mechanisms repairing or minimising increases in oxidative damage (e.g. Folkvord et al. 2014). Similarly, time budgets have to alter as more time has to be spent foraging at the expense of reproduction

and present further problems by increasing the amount of time when they may be more susceptible to predation or parasites (Pitcher et al. 1988).

As mentioned earlier, the temperature experienced during development can have lifetime consequences for an organism by fixing phenotypes during ontogeny (Atkinson 1996). Furthermore, the timing and sequence of physiological events can be altered by thermal exposure during development (Spicer et al. 2011). Martell et al. (2006) exposed haddock (*Melanogrammus aeglefinus*) embryos to a range of temperature and observed changes in the onset of tissue development as well as the relative order of developmental events. It is unclear what the long term effects of such changes were, yet as the timing and duration of notochord, eye and neural tube development were affected it is likely that there are fitness implications at some stage.

Environmental sex determination, whereby sex is established by an environmental cue during development, has been recorded throughout ectotherms although it is most widely reported for reptiles. The operational sex ratio is affected by temperature (presumably) in order to maximise maternal reproductive fitness based on current environmental conditions (Warner and Shine 2008). Sex ratio not only has a consequence for the mother's fitness but can lead to selective pressure on offspring towards, for example, larger body size (Olsson et al. 2011, Chuche and Thiery 2012). Temperature dependent sex determination (TSD) has been recorded for some fish species (see Ospina-Álvarez and Piferrer 2008, Shen and Wang 2014) including aquarium strains of guppies (Karayucel et al. 2006, Kudo and Karino 2011) with the proportion of males increasing with elevated

temperatures. Whether this is strictly TSD or a combination of genotypic sex determination and temperature effects, however, requires further study.

The duration of development is generally shorter in hotter conditions as the temperature dependence of biochemical reactions result in faster growth (Berrigan and Charnov 1994, Sibly and Atkinson 1994). This relationship extends post development and hotter temperatures results in smaller sizes at maturity. The effect is that species in colder environments take longer to reach maturity but the increase in body size results in greater fecundity and larger offspring size and represents a life history trade-off between current and future reproduction (Atkinson and Sibly 1997). Within a non-stressful temperature range, the 'Temperature-Size Rule' (Atkinson 1994, 1996) is one of the most taxonomically widespread rules in biology (Angilletta 2009) although the counter-intuitive idea that faster growth should lead to a smaller size at maturity has been difficult to explain (Angilletta et al. 2004, Kingsolver and Huey 2008).

As a general rule, being larger has greater fitness benefits through increasing fecundity, survival and success in competitive interactions (e.g. Kingsolver and Huey 2008, Brandner et al. 2013). Furthermore, performance at the optimal temperature is arguably better at higher temperatures however hotter temperatures during development lead to smaller body sizes (Kingsolver and Huey 2008). Ultimately, the contribution to an individual's fitness of these rules depends on the level at which they are recorded and the performance trait measured. For example, shorter development time results in a smaller body size yet may increase an individual's number of reproductive encounters, allow for greater access to

resources and decrease a population's generation time, all of which have implications on the speed at which adaptation can occur (for reviews, see Roff 1980, Gotthard and Nylin 1995, Dmitriew 2011).

Growth rate and maturation post development can be further impacted by environmental temperature. Age at maturity has been closely linked to temperature controlled growth rate (e.g. see Stearns and Koella 1986) although there is evidence to suggest that temperature can have a direct effect on maturation (Dieckmann and Heino 2007, Kuparinen et al. 2011). Organisms can also overcome periods of suppressed growth through compensatory growth (Ali et al. 2003, Auer et al. 2012). Once the factor suppressing growth, be it temperature or food, is ameliorated, growth can be accelerated through increased food intake so that maturity occurs at a similar size to non-stressed conspecifics (Auer et al. 2010, Dmitriew 2011). Compensatory growth can, however, carry costs such as a decline in offspring production, lifespan and other fitness traits (see Mangel and Munch 2005).

Once mature, the fundamental effect of environmental temperature continues to influence physiological processes by altering swimming performance (e.g. Wilson 2005, Hein and Keirsted 2012), energetic demands (e.g. Morgan and Metcalfe 2001) and digestion and assimilation efficiency (McConnachie and Alexander 2004, Secor and Boehm 2006) . Temperature also has direct effects on gonadal maturation (Dorts et al. 2012) and sex steroids which are essential for controlling ovulation, oocyte size, fertilisation rate, oogenesis and spermatogenesis (Arantes et al. 2011, Miranda et al. 2013). Behavioural and temporal patterns such

as activity patterns (Reebs 2002, Mehner 2012), switching between diurnal or nocturnal feeding (Fraser et al. 1993) time budgets (Valdimarsson and Metcalfe 1999, Rensing and Ruoff 2002), predator avoidance behaviour (Greenwood and Metcalfe 1998, Weetman et al. 1998, 1999), spawning activity (Soria et al. 2008) and aggressive behaviour (Biro et al. 2010) are also all affected by changes in temperature.

Thermal niche and geographic range

The effect of temperature on life history variables can be viewed as a series of trade-offs. Either at a whole body level between offspring size and number (Smith and Fretwell 1974, Collin 2012); size and age at maturity (Atkinson 1994, Sibly and Atkinson 1994); time spent foraging and reproducing (Folkvord et al. 2014) or at a cellular/physiological level (Angilletta et al. 2003). Metabolism uses oxygen and food reserves to fuel physiological activity and so an increase in metabolism, caused by increasing temperature, leads to a higher oxygen demand and greater respiratory activity. Physiological costs such as reduced mitochondrial efficiency (Abele et al. 2002), an increase in reactive oxygen species (ROS) which are toxic byproducts of metabolism and, ultimately, a reduction in lifespan (Finkel and Holbrook 2000) have wide ranging impacts on form, function and behaviour.

The effect of these trade-offs are apparent when studying latitudinal or altitudinal clines of species and are particularly informative about species' adaptive responses to the selective pressure placed on them by temperature (see Roff 1980, Gotthard and Nylin 1995, Gaston and Spicer 2001). For example, in a study of the butterfly *Aricia agestis* in the UK, Burke et al. (2005) demonstrated

that the butterflies at the warmer edge of their range generally had a shorter development time and consequently lower pupal weight and adult final size in order to complete two generations per year. Butterflies at the colder edge had a longer generation time such that they could only complete one generation within a year. Similarly, the eastern mosquitofish (*Gambusia holbrooki*) has expanded its range across Europe after being introduced into Spain in 1921 and Italy in 1922 (Vidal et al. 2010). Within Spain, at the edge of their invaded range, southern populations invest more energy in reproduction but achieve a smaller final size than populations further north (Benejam et al. 2009).

The combination of a species' life history response to temperature and evolved thermal tolerance (as well as precipitation for terrestrial ectotherms) is integral in determining an individual's geographic range extent (Kingsolver 2009, Calosi et al. 2010). The potential thermal range an individual can inhabit is truncated, however, through a combination factors such as resource competition, predation and physical boundaries. The result is that individuals generally inhabit a *realised thermal niche* which is smaller than their actual thermal tolerance would allow (Magnuson et al. 1979). The *fundamental thermal niche* comprises the range of temperatures a species can theoretically exist within and needs to be determined either by lab experiments or observed as a consequence of range shifts or changing environmental conditions (Magnuson et al. 1979). Species with broader fundamental niches are generally more likely to survive in wider range of environments, in greater densities and consequently have wider geographical ranges (Gaston and Spicer 2001).

Determining a species' fundamental niche is important for understanding the potential range extent a species could inhabit if physical or biological barriers were removed. Many studies have attempted to determine fundamental thermal niche by recording performance traits over a continuous range of temperatures (see data within Deutsch et al. 2008). However, as wild species rarely experience constant temperatures, nor solely mean ambient or maximum temperatures the results could be misleading or inaccurate (Clusella-Trullas et al. 2011, Paaijmans et al. 2013). Costs associated with plasticity mean that a species' response will differ when exposed to stable temperatures or ecologically realistic fluctuating temperatures (Angilletta 2009, Bozinovic et al. 2011).

Fluctuating thermal conditions (which have the same mean temperature as a constant temperature) has a variety of molecular, philological and life history effects. These include increased metabolic rate (e.g. Lyytikainen and Jobling 1998); shorter development, poorer condition at metamorphosis but greater jumping performance (e.g. Niehaus et al. 2006); improved locomotor performance (e.g. Měráková and Gvoždík 2009) and reduced age and length at maturity (e.g. Dhillon and Fox 2007). One of the most profound effects of fluctuating temperatures has ecological and evolutionary consequences by increasing the breadth of thermal tolerance (e.g. Schaefer and Ryan 2006, Folguera et al. 2009, Arias et al. 2011) and selecting for thermal generalism (Ketola et al. 2013).

The actual characteristics such as the mean, breadth and predictability of the fluctuations are also important, however, and each can lead to different effects on fitness traits. Moderate fluctuations had similar effects on heat shock protein

expression and metabolic rate but both increased significantly when tested in high variability treatments (Folguera et al. 2011). Similarly, larger thermal fluctuations lead to a higher CT_{max} (Clusella-Trullas et al. 2011). Měráková and Gvoždík (2009) found that the swimming speed of newts that were subjected to low, medium or high thermal variability as eggs was highest in the most variable treatment group. Other studies found that the extent of thermal variation as a combination with overall mean temperature was important for determining performance traits such as thermal tolerance (Terblanche et al. 2010), survival and population growth (Bozinovic et al. 2011) as well as development and growth rate (Arrighi et al. 2013).The irregularity of fluctuation can also have an important effect on traits such as raising the CT_{max} and increasing body size compared to both constant and predictably repeating cycles of temperature (Schaefer and Ryan 2006).

Interestingly, a study by Fischer et al. (2011b) also demonstrated that fluctuating temperatures were associated with shorter development times, increased heat but decreased cold stress resistance, decreased heatshock protein expression, and increased immunocompetence in a butterfly species compared to stable temperatures. However, for eight of the nine traits they measured, the direction of the effect was in the same direction for both stable and fluctuating temperatures, for example heat tolerance was higher at higher stable and fluctuating temperatures. The conclusion being that although studies using fluctuating temperatures will be more ecologically accurate the broad conclusions about the effect of temperature on phenotypic traits would be the same if stable temperatures were used.

Why the interest in temperature?

It is clear that temperature has a huge role in all aspects of ectotherm biology and is an important vehicle for investigating environmental adaptation from a molecular to macroecological level. Over recent decades, however, there has been a greater focus into the consequences of human-induced rapid environmental change (HIREC) such as the effects of climate change, the increased occurrence of extreme weather events and invasive spread (Sih et al. 2011, Sih 2013).

Climate change and extreme events

The Intergovernmental Panel on Climate Change (IPCC) predicts that, depending on the model scenarios, there will be a global mean temperature increase of between 1.5 and 4^0C by the end of the century (IPCC 2014). Coinciding with the increase in temperature is an increase in unpredictable extreme weather events such as heatwaves, storm surges and increased rainfall that operate on a much more rapid timescale than global temperature increase (Schar et al. 2004, Peterson et al. 2013).

Changing climate conditions will have fundamental effects on species and result in numerous consequences encapsulated by three broad categories: changes in phenology (Bradshaw and Holzapfel 2010, McNamara et al. 2011); distributions (Parmesan and Yohe 2003, Lauzeral et al. 2011); and a reduction in body size (Gardner et al. 2011). Species level changes will lead to further disruption in ecosystem functioning as species move into or out of habitats and predators and their prey species adjust to changing conditions at different rates (e.g. Ferris and Wilson 2012, Grigaltchik et al. 2012).

Invasive spread

Changing conditions may facilitate range spread, especially for species whose realised niche is smaller than their fundamental niche (Lauzeral et al. 2011). The organisms most likely to be able to withstand climate change are also the ones with the capabilities to be successful invasive species.

Invasive species are cited as one of the most serious causes of global biodiversity loss (Clavero and Garcia-Berthou 2005, Pimentel et al. 2005). During invasion events, introduced species become exposed to novel abiotic and biotic conditions which invariably lead to a failure to colonise (Reznick and Ghalambor 2001). Invasions that are successful, however, are generally made by the more phenotypically plastic organisms (Ghalambor et al. 2007, Engel et al. 2011). Once introduced into a novel habitat the invaders have a number of direct and indirect impacts in the new environment affecting the indigenous inhabitants and numerous ecological processes (Miner et al. 2005, Ehrenfeld 2010). In the USA, for example, non-native species are considered the cause of risk for 42% of the species listed as Threatened or Endangered (Pimentel et al. 2005).

The consequences of climate change and invasive spread are manifold. Ecologically, ecosystem dynamics will be altered through the disruption of predator-prey relationships, changes to phenology and biodiversity loss (Ricciardi et al. 2013, Simberloff et al. 2013). There are also anthropocentric consequences as food security is threatened and ecosystem services are altered with unknown consequences (Brierley and Kingsford 2009) as well as the significant financial ramifications. The cost of mitigating effects and managing the consequences of

over 500 non-native species costs the British economy around £1.7 billion each year (Williams et al. 2010) while the 50 000 invasive species in the USA costs around $120 billion. There are other complications as changes in diversity will lead to a localised financial loss through detrimental effects on wildlife tourism or angling as species change location or are regionally extirpated (Dudgeon et al. 2006, Strayer and Dudgeon 2010).

Identifying and predicting effects

Because of the biological and financial costs connected to climate change and biological invasion it is becoming increasingly important to predict the potential consequences (Clusella-Trullas et al. 2011, Somero 2011). Modelling approaches, however, require a developed understanding of species' behavioural, physiological and genetic vulnerability to environmental change (Huey et al. 2012). Similarly, the effective management of invasive species also improves with increasingly detailed knowledge on the biology of invasive species (Matthews and Marsh-Matthews 2011).

Trait based studies operate at an organism level (e.g. Janion et al. 2010, Miranda et al. 2013) and across geographical gradients (Benejam et al. 2009, Calosi et al. 2010). The data are used to indicate the potential responses to changing conditions at a local scale or explain macrophysiological patterns over large geographical and temporal scales (Gaston et al. 2009, Angert et al. 2011). This information can then be used to understand the causes and evolutionary consequences of physiological variation; model the potential outcomes of changing

conditions; and ultimately develop management strategies (Kearney and Porter 2009, Rowland et al. 2011).

The Trinidadian guppy (*Poecilia reticulata*) – Model species

The guppy (*Poecilia reticulata*) is a tropical, livebearing fish endemic to the islands of Trinidad, Tobago and northeast South America. Widespread in fresh, and some brackish, waters guppies are omnivorous, opportunistic feeders capable of tolerating turbid and polluted habitats (Houde 1997, Magurran 2005).

The use of guppies in scientific research dates back to the early 20th century with the work of Johannes Schmidt and Øjvind Winge among others (e.g. Schmidt 1919, Winge 1922). It was, however, the mid-century work on sexual traits and behavioural ecology by Clark and Aronson (Clark et al. 1948, Aronson and Clark 1952), the Haskinses (Haskins and Haskins 1950) and Baerends and co-workers (Baerends et al. 1955) that identified the guppy as an excellent model species.

Guppies are hardy, robust to manipulation and readily adapt to laboratory aquaria. Accordingly, over the past 60 years the guppy has become an important model species for investigating questions about evolutionary ecology as well as diverse topics such as the evolutionary impact of fisheries (Reznick and Ghalambor 2005), idiopathic scoliosis in humans (Gorman et al. 2010) and toxicity testing (Schafers and Nagel 1991). The resilience of guppies to experimental conditions also enables researchers to make *whole-animal* measures of fitness which can be more readily associated with ecological consequences than cellular or tissue based approaches (Huey and Stevenson 1979).

Image 1: Mature male (left, top right) and female (bottom right) guppy, image by Sean Earnshaw.

The upstream, low predation – downstream, high predation system

In Trinidad, guppies are prevalent throughout freshwater and some brackish habitats and have shown to be remarkably flexible in their behaviour and morphology. Sexual selection figures highly in guppy populations. Males exhibit a complex colour pattern often highly variable between individuals but well conserved through genetic inheritance (Endler 1980) whereas females are generally monochrome and dull in colour **(Image 1)**. Mating behaviour is characterised by two tactics; the first involves a well described sigmoid courtship display which either elicits a consensual response from the female or is completely ignored. The second is a coercive technique termed gonopodial thrusting (or "sneak mating") whereby the male approaches the (generally unreceptive and unresponsive) female from behind and attempts to insert his gonopodum into the female's genital aperture (Houde 1997). Sneak mating transfers fewer sperm than consensual copulation however still results in successful insemination (Pilastro and Bisazza 1999, Matthews and Magurran 2000).

The rivers flowing through the range of hills in the North of Trinidad are broadly defined by cooler, clearer, fast flowing headwaters becoming warmer, slower and more turbid as they enter the lowlands. These rivers are periodically punctuated by waterfalls which act as natural barriers allowing for only downstream migration and preventing upstream access by predators. Along this gradient, guppies from headwaters are commonly larger, have limited schooling and anti-predator behaviour, female biased sex ratio and with males exhibiting bright colour patterns. Downstream, populations are characterised by smaller

individuals, an equal sex ratio, less colourful males and a more developed response to predators (Seghers 1974, Liley and Seghers 1975).

One-way migration results in upstream populations that are generally low in fish diversity and predators (typically only *Rivulus hartii* a small killifish which preys on small or juvenile guppies, (Fraser et al. 1995). Downstream, the piscivorous cichlid *Crenicichla alta* and characins *Astyanax bimaculatus* and *Hoplias malabaricus* and other potential guppy predators become increasingly common (Magurran et al. 1995). As with many of the guppy's life history responses these behavioural and morphological changes along the water course are attributed primarily to the selective influence of predators (Reznick et al. 1990).

A transplant experiment which relocated fish from areas of high to low predation demonstrated that within just two years of introduction guppies had the appearance of those from low predation environments (Endler 1980). Further study went on to show that high predation populations matured at smaller sizes, produced more and smaller offspring and allocated more of their energy to reproduction than their low predation counterparts and that there was a genetic basis to this response (Reznick 1982a, Reznick 1982b).

Although the effect of predation has a marked and unequivocal effect on shaping guppy life history it is important to distinguish its effect from the myriad environmental variables that are closely interlinked. Upstream, low predation environments are also commonly cooler, faster flowing, more shaded and less productive than the downstream, high predation, equivalents. Between these extremes there is also a continuum variety of habitat types and conditions causing

differences in population size and organismal diversity as a result of the quick changes in slope and resulting waterfalls.

Studies investigating abiotic effects on biological and life history characteristics of guppies indicate that predation may not be the singular reason for the evolution of life history traits (Grether et al. 2001b, Reznick and Ghalambor 2001, McKellar et al. 2009).

The effects of temperature on guppy biology

Liley and Seghers (1975) reported the water temperature of 23 locations in Northern Trinidad from 24.3-26.2^{0}C in spring waters to 26.9-29.1^{0}C in lowland rivers, a range of nearly 5^{0}C. Over a two year period (Alkins-Koo 2000) recorded the monthly temperature of two southwestern streams finding water temperature to range from 20-28^{0}C with occasional diurnal changes of up to 7.5^{0}C during the dry season while the Pitch Lake population of guppies, also in the southwest, experience temperatures exceeding 40^{0}C (Pettersson et al. 2004).

Despite the fundamental effect of temperature on ectotherms, the gradient in temperature experienced by guppies along Trinidadian waterways, and the ubiquity of guppies in lab aquariums there is a relatively small body of work investigating the effect of thermal conditions on the physiology and life history of guppies.

Gibson (1954) reported the upper lethal temperature for guppies to be 32^{0}C with males particularly affected by elevated temperatures. This temperature

has been revised upwards by (Chung 2001) who determined the CT_{max} to be between 38.9 – 40.6^{0}C depending on acclimation temperature.

Gibson and Hirst (1955) provide some of the first measures of guppy growth at five temperatures between 20^{0}C and 32^{0}C. Fish reared at 23^{0}C and 25^{0}C exhibited the fastest growth and males matured sooner than in the 20^{0}C and 30^{0}C treatments. At 32^{0}C, however, fish grew slowly, developed deformities and males and females remained indistinguishable by 110 days indicating 32^{0}C is detrimental to growth and maturation.

The effect of population origin on juvenile growth was investigated by (Liley and Seghers 1975). Using guppies from an upstream (low predation, larger fish, more colourful males) and downstream (high predation, smaller and less colourful fish) population juvenile growth was recorded at both 23 and 28^{0}C in the lab until 19 weeks of age. Females continued to grow throughout the experiment while males from both populations and temperatures grew rapidly until maturation at 9-11 weeks at which point growth rate dropped off. Males and females from both populations grew larger in the lower temperature treatment. Males from the upstream population were consistently larger than their downstream counterparts regardless of temperature whereas downstream females were larger than their upstream reciprocals at both temperatures but not significantly so. This suggests that growth has both an environmental as well as genotypic component.

Weetman and coworkers (Weetman et al. 1998, 1999) investigated the shoaling response of guppies at different temperatures. Shoaling increased with

increasing temperature but that the effect was strongest at higher temperatures and in the presence of a predator. Temperature alone was not responsible for the shoaling effect however the response and allocation of time budget is temperature dependent as feeding requirements increase (and so exposure to predators) with increasing temperature.

Recently, Muñoz et al. (2012) exposed guppies to a range of temperatures and recorded body mass, burst swimming speed and resting metabolic rate (RMR). Temperature had no effect on burst speed but higher temperatures led to lower body mass and higher RMR and the authors concluded that temperatures >30^{0}C may be too high for guppies to tolerate. In a second study in the same lab and using the same temperature range, Breckels and Neff (2013) demonstrated that males produced shorter and slower sperm in hotter temperatures although immunity and brood survival was unaffected. These results underscore the lack of clarity in the effects of temperature on guppy biology.

For the closely related Poeciilid fish *Gambusia holbrooki* thermal environment has a marked effect on life history traits (Meffe 1990, 1991, 1992), behaviour (Wilson 2005) and physiology (Wilson et al. 2007a). Temperature has also been cited as causing evolutionary responses in populations within 60 years of introduction to a novel environment (Stockwell and Weeks 1999, Stockwell and Vinyard 2000). Like the guppy, *G holbrooki* has a high reproductive potential, is phenotypically plastic and very hardy having been recorded in temperatures ranging from 0-45^{0}C, salinities from 0-41ppt and degraded habitats (see review by Pyke 2008 and references therein).

With other Poeciliids exhibiting strong responses to temperature and multiple biotic and abiotic factors having a pronounced effect on guppy life history, it is not a great leap to suspect that environmental temperature will have consequences for guppies too.

The guppy as an invasive species

Guppies have been a popular feature in aquariums and laboratories throughout much of the 20th and 21st centuries. Consequently, accidental escapes and orchestrated releases (largely in attempts to control mosquitos) have led to the guppy becoming established in at least 72 countries outside of its native range (Deacon et al. 2011). In a number of these countries it has been observed that guppies have a negative impact on local fish assemblages by out-competing them for space and resources while their efficacy as a mosquito control agent varies is unclear (Deacon 2010).

A feature of guppies both in Trinidad and abroad is their ability to adapt to novel and sub-optimal environments. In lab experiments guppies have been shown to tolerate a broad range of environmental conditions including temperature (Gibson and Hirst 1955, Chung 2001) and salinity (Gibson and Hirst 1955, Chervinski 1984) and *in situ* studies have shown the guppy to be tolerant of and abundant in degraded habitats and poor environmental conditions (Winemiller et al. 1990, Casatti et al. 2006). Many studies have been shown guppies to be phenotypically plastic (e.g. Reznick and Bryga 1987, Reznick 1990, Kelley et al. 2003, Magellan et al. 2005). This flexibility, in combination with an omnivorous feeding strategy (Dussault and Kramer 1981), capacity for rapid life history

evolution (Reznick et al. 1990), plus the ability of a single female to establish a sustainable population (Carvalho et al. 1996, Deacon 2010) means that guppies have many of the characteristics identified as important for enabling successful invasion (Garcia-Berthou 2007).

Recent studies using transplant and mesocosm experiments have demonstrated that the local adaptions by guppies have the capacity to cause ecosystem-level effects and be a potential driver of evolutionary change (Palkovacs et al. 2009, Bassar et al. 2010). Consequently the effect of a successful colonisation has eco-evolutionary consequences for the guppies, the invaded ecosystem and the organisms and processes therein (Palkovacs et al. 2009).

For freshwater fish, introduction into new environments often requires anthropogenic assistance (Reznick and Ghalambor 2001). Once present in the novel environment, the abiotic conditions faced are considered key to the success or failure of an invasion (Moyle and Light 1996a, b). The ability of guppies to persist despite widely differing thermal regimes that exceed the range of temperatures encountered in Trinidad appears a critical step to their successful establishment (for example at a site where guppies are present in central Mexico, over a two week period water temperatures varied between 17ºC and 21ºC, **Appendix 1**).

The guppy's ability as an invasive species is well documented and temperature is one of the main factors restricting north- and southward range expansion and establishment. However, with the threat of regional and global climate change the potential for further expansion becomes greater. It is therefore

important to develop an understanding of a species' fundamental thermal niche to enable predictions of the possible effects (Pörtner and Knust 2007, Somero 2011, Diamond et al. 2012). Furthermore a good knowledge of the guppy's thermal ecology is essential if environmental managers are to be able to mitigate any negative effects of present and future invasions (e.g. Laha and Mattingly 2006, Priddis et al. 2009, Warren et al. 2013).

Question

To predict the potential consequences of changes in thermal regime it is first necessary to investigate how species respond to ecologically relevant changes to the thermal environment (Somero 2011, Chown 2012, Diamond et al. 2012). Therefore, the question in which this thesis is interested is:

How do changing environmental conditions affect fitness-altering traits?

In the following five chapters I will describe the investigations I undertook in an effort to develop an enhanced understanding of the guppy's thermal niche.

Chapter Two

Temperature and light both play a key role in controlling daily activity patterns as temperature has a direct effect on ectotherm metabolism and brighter conditions increases the possibility of being detected by predators. In wild fish, light has a proven effect on activity while temperature has been less studied. As temperature and light are so closely connected, in an attempt to disentangle the effects of light and temperature from time of day, I investigated the relative importance of each factor on patterns in male mating activity during different periods of the day. The prediction in this experiment was that mating attempts would be lowest when light and temperature at their highest (midday).

How does natural variability in abiotic conditions affect daily patterns of mating activity in wild fish?

Chapter Three

The temperature of water in natural systems is subject to short term variability through a variety of sources (e.g. rainfall, insolation, industrial water effluent). The breadth of temperatures that a species can withstand for short periods of exposure provides a measure of their thermal tolerance. To investigate the effects of short term thermal change I exposed individual guppies to a change in temperature over a broad thermal range. I then created thermal performance curves (TPCs) to describe the effect on male mating activity. I hypothesised that the guppy's thermal breadth would be similar to the water temperatures they experience in Trinidad.

What are the immediate effects of temperature change on mating behaviours?

Chapter Four

Whether organisms can respond to prolonged periods of thermal change without a drastic loss of performance depends upon their ability to acclimate to the novel temperature. The success of acclimation is therefore dependent on an individual's phenotypic flexibility. To investigate the temperature range over which guppies can acclimate completely I created three thermal regimes and allowed male and female guppies to acclimate for over 60 days. I then recorded the effect on male mating activity, observed changes in reproductive output and measured growth parameters in offspring born during the acclimation period. My prediction was that guppies would successfully acclimate and thus display highest performance when tested in the temperatures in which they were acclimated.

How does thermal acclimation alter performance in mating behaviour, growth and offspring traits at different temperatures?

Chapter Five and Six

Undertaking investigations using stable temperatures removes a source of variability from the analysis however questions remain over the ecological relevance of the results as organisms rarely experience stable temperatures in the wild. To investigate how temperature alters life history in environmentally realistic thermal conditions, I created two thermal regimes that fluctuated over daily cycles similar to those of the guppy's natural environment. With individually-housed fish I first recorded the effect of thermal regime on life history traits over two generations. Using the same experimental set-up I then investigated a number of traits linked to male reproductive success – mating behaviour, sperm length and body colouration. Based on theoretical predictions, I hypothesised that the warmer treatments would result in faster growth, smaller adult size, decreased offspring size, shorter sperm and less colour than the colder treatment.

What are the effects of environmentally-realistic fluctuating temperatures on life history traits?

What are the effects of environmentally-realistic fluctuating temperatures on sexually selected traits?

Aims

The aims of this thesis are to:

- Describe how natural variability in abiotic conditions affects daily patterns of mating activity in wild fish. **Chapter Two**
- Establish what the immediate effects of temperature change are on mating behaviours. **Chapter Three**
- Investigate how thermal acclimation alters performance in mating behaviour, growth and offspring traits at different temperatures. **Chapter Four**
- Explore what the effects of environmentally-realistic fluctuating temperatures are:
    - on growth and reproductive characteristics. **Chapter Five**
    - and on sexually selected traits. **Chapter Six**

Chapter two: Interplay of temperature and light influences daily mating activity

: water temperature in Trinidad is more variable than expected; and this variability influences guppy activity patterns… #WildEnvironment

Lay summary

As a general pattern, temperatures are relatively stable at the equator and increase in variability with increasing latitude. This rule results in species found at higher latitudes having a greater thermal tolerance range than low latitude counterparts. I recorded the water temperatures experienced by guppies in three Trinidadian streams and found them to follow a variable daily pattern with a temperature range of up to 7^0C each day. This variability in temperature is likely to have been an important factor contributing to guppies developing a broad temperature tolerance range which has enabled them to successfully invade other environments. In this experiment I observed guppy mating behaviour at dawn, noon and dusk over successive days to assess the effects of temperature, light and time of day on activity patterns. I found that time of day modified the effect of temperature and light and that regardless of conditions male guppies were most active during dawn. I conclude that variability in temperature is an important behavioural driver and should be incorporated into future ecological studies of wild guppies.

Abstract

In this study I investigated the environmental regulation of daily mating activity of guppies (*Poecilia reticulata*). I observed male guppy mating behaviour for 17 days over three time periods: dawn, noon and dusk, while recording natural variation in water temperature and light level. Water temperature was highly variable on a daily basis (ranging up to 7^0C each day) and there was a recurring pattern in daily variation for both light and temperature. Activity was highest at dawn and similarly low during noon and dusk although mating behaviour continued throughout the day. Mixed effects models indicate that light and temperature affect mating behaviour differently at different times of the day and can also have opposing effects. I suggest that the environmental heterogeneity of Trinidad's streams has led to a broad thermal tolerance and contributed to the guppy's high level of phenotypic plasticity and its success as an invasive species. Furthermore these results show that daily variation in temperature and its interaction with light should be considered in future studies of guppy mating behaviour.

Keywords

Daily activity – environmental variability – temporal patterns – mating behaviour – tropical freshwaters – invasion biology

Introduction

Individuals face the challenge of maximising their fitness when exposed to predictable changes in environmental conditions. Seasonal changes in day length and temperature are important for the phenology of seasonal responses such as flowering in plants (Andres and Coupland 2012), reproductive diapause in butterflies (Goehring and Oberhauser 2002), shell morphology in snails (Kohler et al. 2013) gonadal maturation and spawning in fish (Pankhurst and Porter 2003), migration in birds (Both 2010) and decisions on energy budget requirements in mammals (e.g. Valencak et al. 2009). On a finer scale, circadian rhythms of behaviour such as daily patterns of predator-prey interactions (Vidal et al. 2011) and of general activity (Iacarella and Helmuth 2012, Ware et al. 2012) are generated endogenously but entrained by external abiotic factors; the two most important being the closely interrelated effects of light and temperature.

Temperature has direct effects for ectotherms at all levels of organisation as metabolic reaction rates are determined by temperature (Hochachka and Somero 2002). Furthermore, processes such as locomotion (Besson and Cree 2010), spawning activity (Soria et al. 2008), feeding and digestion (Secor and Boehm 2006) depend on different functional systems and may therefore have different optimal temperatures (Angilletta 2009). Behavioural thermoregulation enables ectotherms to optimise metabolic processes (Bicego et al. 2007) as they seek out preferred temperatures to maintain body temperatures and improve performance (e.g. Kiefer et al. 2006, Speed et al. 2012). Many fish species in thermally stratified habitats undertake daily vertical migrations in order to maximise bioenergetic

efficiency: during the day, light intensity is typically the primary trigger to begin migration whereas water temperature and hydrostatic pressure guide position during the night (reviewed in Mehner 2012). This selective positioning results in improvements in performance, such as increased digestive efficiency (Wurtsbaugh and Neverman 1988, Neverman and Wurtsbaugh 1994) or reduced swimming costs (Ohlberger et al. 2008) although increases the likelihood of becoming parasitized or predated upon (Angilletta 2009).

When the habitat does not offer enough spatial thermal heterogeneity, ectotherms can thermoregulate temporally by taking advantage of natural diurnal variability in temperature and adjusting activity patterns over the course of the day. In these cases, behaviours and biological functions such as digestion and swimming performance could be timed to coincide with the time of day when conditions are most favourable (e.g. Greenwood and Metcalfe 1998, Johnston et al. 2004, Railsback et al. 2005).

As a general pattern, the thermal tolerance range of ectotherms is greater with increasing latitude (Sunday et al. 2011). In tropical regions, water (and air) temperature is considered to be relatively stable with only limited daily and seasonal variability (Sunday et al. 2014) and this has resulted in a larger portion of high latitude species with narrower thermal tolerance than found in higher latitudes (Tewksbury et al. 2008). Accordingly, much of the research focus in tropical freshwaters has been concerned with other abiotic and biotic factors such as the importance of daily cycles of light and predation while the effect of temperature on patterns of fish activity has been little studied (Reebs 2002).

However, there is evidence to demonstrate that daily temperature oscillations in tropical freshwaters may be greater than previously considered and therefore an important driver of behaviour.

Guppies (*Poecilia reticulata*) are freshwater fish native to Trinidad and Tobago and northeast South America although they have successfully established populations in every continent except Antarctica (Deacon et al. 2011). In their natural range, variation in light and predation risk have resulted in adaptive selection in reproductive behaviour and life-history traits (e.g. Reznick and Endler 1982, Endler 1987). Light is essential for multiple activities such as foraging and communication, in particular being able to see, and be seen by potential mates (Endler 1995, Gamble et al. 2003, Chapman et al. 2009). This is especially important for male guppies who perform display behaviours whilst exhibiting their colour patterns to attract females (Houde 1997). Studies have shown that the intensity of courtship is higher during dim light conditions experienced earlier or later in the day than in the very high light levels experienced at midday (Endler 1987, Reynolds et al. 1993, Long and Rosenqvist 1998). The guppy's alternative mating strategy is a non-consensual 'sneak mating' whereby the male approaches the female from behind and attempts to inseminate her by thrusting his gonopodium into her genital pore. 'Sneak mating' is not closely associated with light (Endler 1987) and appears a highly variable, opportunistic tactic evolved as an adaptive response to predation (Magurran and Seghers 1994) rather than any single abiotic condition.

While a few studies have explored the association between temperature and guppy behaviour (Johansen 1985, Weetman et al. 1998, 1999) until recently (Muñoz et al. 2012, Breckels et al. 2013, Breckels and Neff 2013) the thermal ecology of guppies remained largely un-investigated. The reason behind this, in part, is because the daily variability of water temperatures within Trinidad is considered to be quite small (e.g. Kenny 1995, Grether et al. 2001b) and therefore other biotic factors (e.g. predation (Reznick and Endler 1982), resource availability (Grether et al. 2001b) and light (Archard et al. 2009)) driving guppy ecology have been more intensively studied. However, water temperatures at a site in the Acono River where guppies are present, taken at 30 minute intervals between Feb 2013 and May 2014 reveal an unexpectedly high level of daily variation in temperature. Temperatures ranged between 23 and 34^{0}C with a largest range of 10^{0}C in any one 24 hour period **(Appendix 1)**. This daily variation in water temperature is thus greater than previously recorded in Trinidad and provides a source of variation that has an unknown contribution to guppy activity. This daily thermal cycle combined with the effects of light are potentially important in explaining activity patterns and may provide the opportunity for temporal thermoregulation of activities. In this experiment, therefore, I ask the question:

How does natural variability in abiotic conditions affect daily patterns of mating activity in wild fish?

To address this question, this experiment investigates variation in male guppy mating activity over three periods of the day – dawn, noon and dusk – in relation to daily patterns of temperature and light. Taking advantage of using both wild-caught fish and the natural abiotic variability of Trinidad my approach

enables me to explore the effects of temperature, coupled with light, on guppy activity patterns and provides the opportunity to investigate temporal patterns in mating behaviour.

Muñoz et al. (2012) demonstrate that at temperatures around 30^0C the metabolic rate of guppies increases to the point where growth and locomotion become limited. Consequently, if temperature is an important driver of daily activity patterns, I hypothesise that mating behaviours will be highest in the morning and suppressed in the middle of the day and afternoon when temperatures are still elevated. Alternatively, if light is the more important abiotic variable I expect mating behaviour to be lowest in the middle of the day, when light level is highest, and higher towards the start and end of the day, when light is lower (see Endler 1987, Archard et al. 2009).

Methods

Fish collection and housing conditions

Fish were collected in June 2012, using a small one-person seine net, from four locations representative of the types of habitat in which guppies occur in Trinidad **(Figure 1)**. Guppies were collected from an upland site in the Acono River, and two downstream localities: Maraval, a lowland tributary of the Caroni drainage, and a lowland drainage ditch in Charlieville on the edge of the Caroni Swamp. The fourth source was Pitch Lake in the south of Trinidad which represents an unshaded site with greater exposure to variability in light and temperature (Kenny 1995). After transportation to The University of the West Indies campus, St Augustine, the fish were housed in 80 litre stock tanks on the roof of the Life Sciences building.

Using a randomised split plot design, a further twenty-four 80 litre plastic observation tanks were arranged on the roof and filled with aged tapwater. River gravel covered the tank bottoms and Canadian pondweed (*Elodea canadensis*) was added to one corner. Each tank was assigned to one of the four populations. Two males and two females were placed in a tank and left to settle for nine days before experimental observations began. Tanks were covered with a large mesh screen to prevent avian predation. Data from six tanks were discarded because of unexplained mortality during the experiment and therefore, only data from 18 tanks were used in the analysis **(Table 1).**

Figure 1: Locations of guppy populations sampled for use in this experiment. Fish were collected from Acono, an upstream river site; Maraval, a lowland tributary of the Caroni drainage; Charlieville, a lowland drainage ditch in on the edge of the Caroni Swamp; and Pitch Lake, an exposed lentic site.

Table 1: Sizes and sources of fish included in the experiment and subsequent analysis.

Population	No. tanks	No. fish	Ave. female size (sd) mm	Ave. male size (sd) mm
Acono	6	12 ♀, 12♂	20.3 (2.9)	17.6 (1.2)
Maraval	5	10 ♀, 10♂	27.4 (2.0)	18.1 (0.9)
Charlieville	4	8 ♀, 8♂	21.0 (1.7)	15.0 (1.4)
Pitch Lake	3	6 ♀, 6♂	24.4 (1.1)	16.9 (0.6)

Both the stock and observation tanks were subject to natural temperature, light and precipitation and guppies fed on naturally occurring organic matter and invertebrates. Temperature dataloggers (MicroLite Lite 5016, Fourier Systems) were placed in six of the tanks to verify that the temperature cycle in the tanks did not differ from the wild pattern for the duration of the experiment **(Figure 2).**

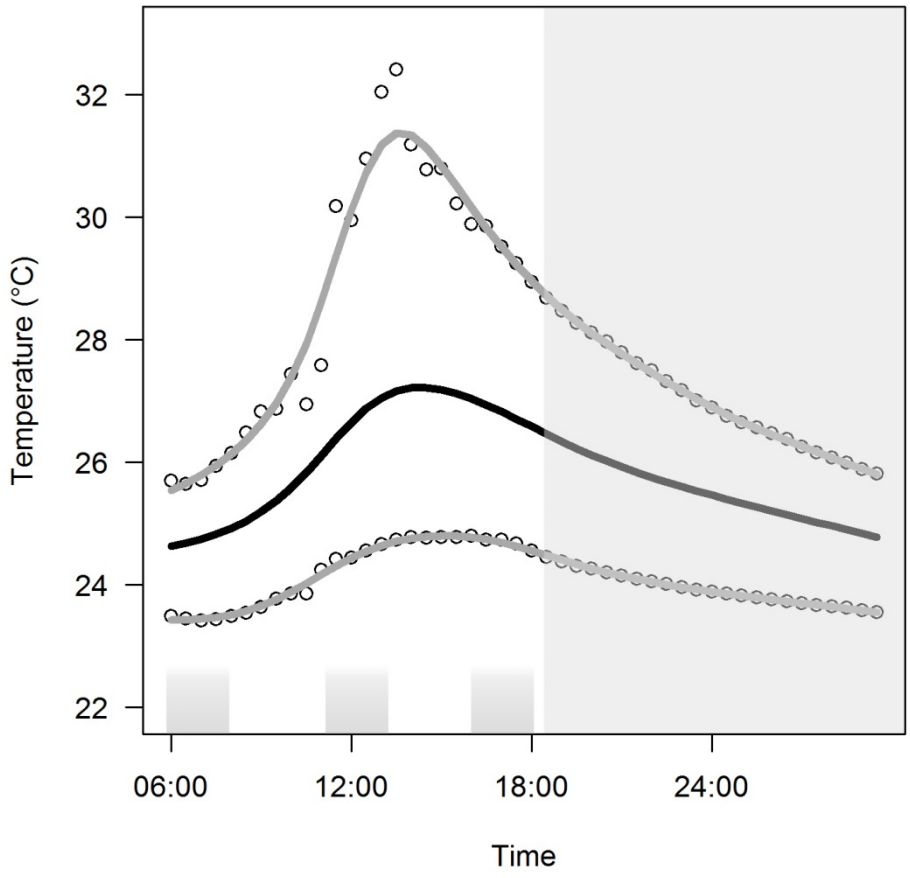


Figure 2 Experimental water temperatures recorded using temperature dataloggers. Black line is the average temperature recorded over 17 days across all tanks at each sampling point, open circles are the maximum and minimum values recorded during the experiment and the grey line is a smooth spline fitted using R. Shaded bands represent observation periods, grey block signifies night time.

Observation protocol

Behavioural observations were made between 30th June and 17th July 2012 during three time periods: 1) '*dawn'*, the two hours following sunrise (c. 05:50); 2) '*noon*', between 11:30-13:30 and 3) '*dusk*' the two hours preceding sunset (c. 18:30) (Fig. 1b). Before each observation event, I recorded the tank's water temperature (to the nearest 0.1^0C) using a digital thermometer, and the amount of light at tank level (lux) using an Extech Hd-450 Lux-Meter (http://www.extech.com/). In total, 825 observation events were used in the analysis.

Individual observations started when the first male in a tank was observed, thereafter becoming the focal male for that session. One male per tank was observed for three minutes in each of the time periods. During each trial, the observer sat in front of a tank and began recording behaviours after allowing the fish to resume normal activity (usually <1 minute). The number of *sigmoid displays* and '*sneak mating*' attempts (gonopodial thrusts) as well as the time the focal male spent *following* a female was recorded using the *JWatcher* behaviour scoring software (www.jwatcher.ucla.edu). The order in which tanks were observed was established using a random number generator.

Statistical analysis

Light measurements were log transformed to make them more normally distributed for all analyses and I assumed that the number of *displays* and '*sneak mating*' attempts followed a Poisson distribution. Comparisons between time periods were performed with one way ANOVA and pairwise Tukey HSD tests for the abiotic conditions, and non-parametric Kruskal-Wallis tests for the *displays* and

'*sneak mating*' behaviours. The relationship between abiotic conditions and mating behaviours were analysed and modelled using a generalised linear mixed effect model (GLMER) from the lme4 package (Bates et al. 2012). For time spent *following* I introduced a hurdle model to analyse non-zero and zero observations separately using the glmmADMB package (Fournier et al. 2012). Hurdle models separate the analysis into two parts: in the first, the data is considered as zeros and non-zeros and a binomial model uses the covariates to model the probability that a zero value is observed. The binary part (non-zero or zero) was modelled by using a logit link function and an exponential distribution (a member of gamma distribution family) was assumed for the non-zero part. This assumption implies that the instantaneous cease ratio of *following* is independent of the duration already spent for *following* (see Cox and Oakes 1984 for a detailed theoretical explanation) but driven by the experiment conditions. In the second part the non-zero observations are modelled using the covariates and a gamma response distribution (Zuur et al. 2009). The binomial component of the model describes the variables that initiate the behaviour while the gamma component describes how the explanatory variables – *light*, *temperature* and *time period* – affect the duration of the behaviour.

Each of the mixed effects models included population, tank and date as random effects; temperature, light and time period were the model's explanatory variables. Multiple models were constructed and model selection was performed using Akaike Information Criterion (AIC) (Akaike 1973). The difference in AIC value for each model (ΔAIC = AIC value – AIC of best model) was quantified with values of two or less indicating acceptable alternative models (Burnham and

Anderson 2001). The AIC weight (w_i) is a measure of the relative likelihood of a model, given the alternatives, of being the best model. Ranging between 0 and 1, AIC weights closer to 1 indicating high support for the model and models with similar AIC weights have a similar level of support in the data (Johnson and Omland 2004).

Results

Each day light and temperature followed their own recurring pattern over the three time periods **(Figure 3).** Light levels ranged from 20 lux in the morning to a peak of 12 190 lux in the middle of the day. Light was highest at noon, lowest at dusk and differed significantly over the day (ANOVA, $F_{2,822} = 285.3$, p-value < 0.001). Pairwise Tukey tests show that light levels differed significantly between noon and dawn (p-value < 0.001), noon and dusk (p-value < 0.001) as well as dawn and dusk (p-value < 0.001, **Figure 3a**).

The lowest and highest temperatures during the experiment were 23.4 and 32.4 ^{0}C recorded during dawn and noon respectively. Temperatures recorded using the dataloggers confirmed that the conditions experienced by the fish in the observation tanks were similar in range, variability and daily cycles to those recorded in nearby rivers where guppies are also present. Temperature differed significantly between time periods (ANOVA, $F_{2,822} = 243.68$, p-value < 0.001, **Figure 3b**) being lowest in the morning, peaking around midday and decaying slowly thenceforth until dawn. Tukey post hoc tests show significant differences between dawn and noon (p-value < 0.001) and dawn and dusk (p-value < 0.001) but not between noon and dusk (p-value = 0.729).

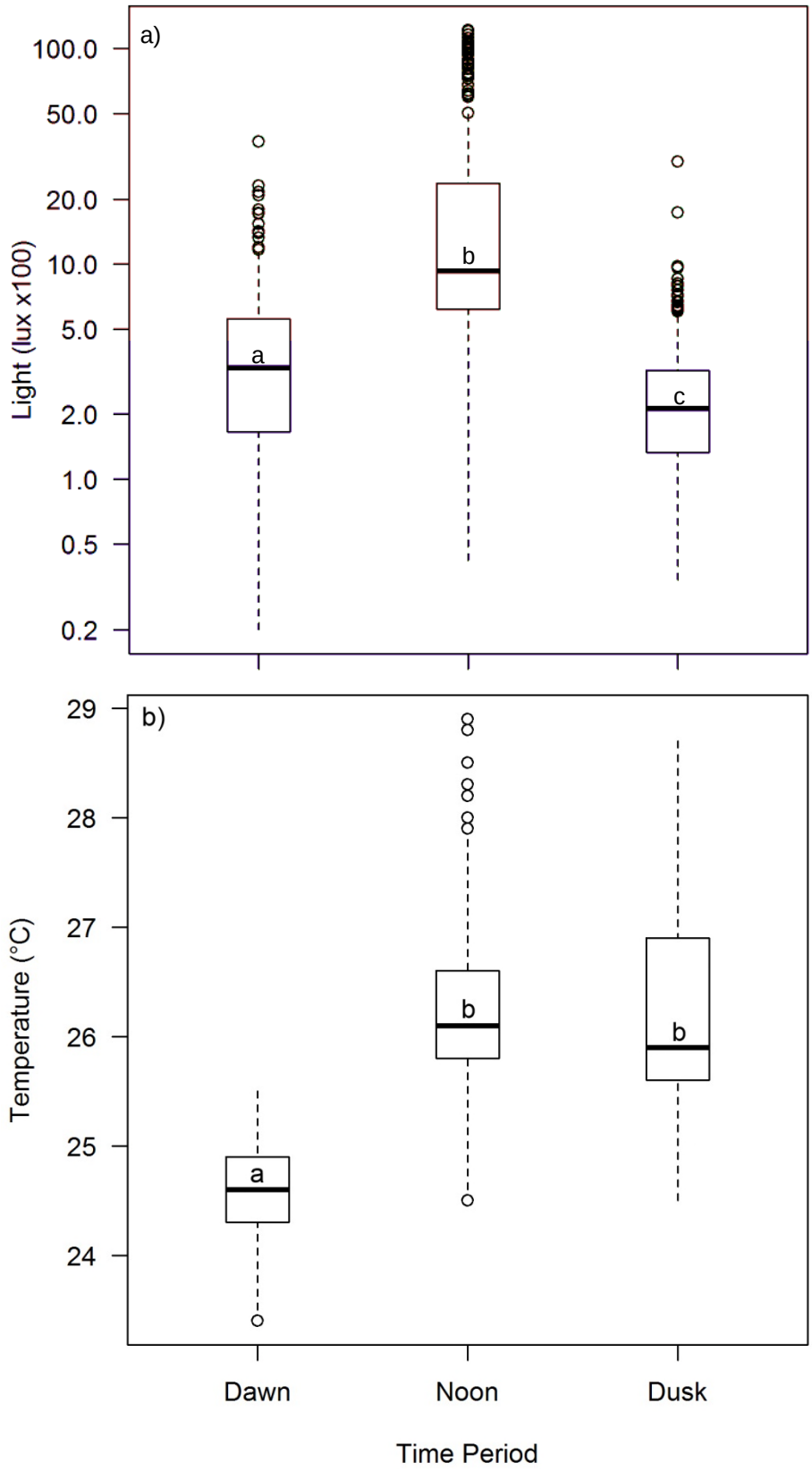


Figure 3: Daily pattern in **a** light and **b** temperature recorded during the experiment. Light was lowest at dawn and dusk and highest at noon, temperature increased from dawn until noon and decreased slowly from then until dawn. Horizontal line shows the median value, top and bottom of the box shows the 75th and 25th percentiles. Dashed line represents the maximum values or 1.5 times the interquartile range of the data (roughly two standard deviations) whichever is smaller (Crawley 2007). The different letters within the boxes mark significant differences (tukey test).

Displays and 'sneak mating'

The number of displays were highest at dawn and differed significantly over the three time periods (Kruskal-Wallis $\chi^2 = 12.09$, df = 2, p = 0.002) with pairwise Kruskal-Wallis tests indicating that the difference in display behaviour was significant between dawn and noon (Kruskal-Wallis chi-squared = 11.63, df = 1, p-value < 0.001) as well as between dawn and dusk (Kruskal-Wallis $\chi^2 = 4.5$, df = 1, p-value = 0.033) but not between noon and dusk (p-value = 0.213). There was no significant difference between time periods in the number of 'sneak mating' attempts (Kruskal-Wallis $\chi^2 = 0.08$, df = 2, p-value = 0.960, **Figure 4**).

A model incorporating light, temperature and their interaction with each of the three time periods, and a Poisson error distribution, was the best model for describing both the number of displays and 'sneak mating' attempts. Over the time periods, males performed more displays to the females during dawn. The number of displays was lowest at noon, when increases in light and temperature had significant opposing effects on mating behaviour. Within periods, light level had a negative effect at dawn and noon but positive at dusk. Conversely, temperature had a negative effect on displays at dawn and a positive effect at noon and dusk **(Table 2, Figure 4)**. Frequency of 'sneak mating' attempts was similar across all time periods. Light had a negative effect on 'sneak mating' attempts at dawn and a positive effect at noon and dusk. Increasing temperature was linked to a reduction in 'sneak mating' attempts at dawn but an increase during noon and dusk **(Table 2, Figure 4)**.

Table 2: Coefficient estimate (standard error) and random effects values for the best fit generalised linear mixed effects models explaining display and 'sneak mating' behaviour and binomial and gamma components of the best fit hurdle model explaining following behaviour.

	Display	**Sneak**	**Following (binomial)**	**Following (gamma)**
Fixed effects:			Estimate (se)	
(Intercept)	11.156 (4.94)	11.861 (5.98)	18.344 (8.65)	3.930 (0.16)
Noon	-16.781 (5.84)	-18.111 (6.55)	-23.37(10.11)	-0.216 (0.20)
Dusk	-7.729 (5.13)	-14.455 (6.11)	-17.324 (9.43)	-0.359 (0.15)
log(Light)	-0.095 (0.08)	-0.179 (0.11)	0.052 (0.15)	-0.071 (0.08)
log(Light): Noon	-0.543 (0.13)	0.098 (0.13)	-0.242 (0.22)	0.023 (0.10)
log(Light): Dusk	0.243 (0.14)	0.224 (0.17)	0.733 (0.31)	-0.068 (0.13)
Temp	-0.495 (0.20)	-0.53 (0.24)	-0.713 (0.35)	-
Temp: Noon	0.701 (0.24)	0.734 (0.27)	0.960 (0.41)	-
Temp: Dusk	0.305 (0.21)	0.58 (0.25)	0.691 (0.38)	-
Random effects:			Variance (sd.)	
Tank	0.864 (0.93)	0.461 (0.68)	0.447 (0.67)	0.23 (0.48)
Date	0.116 (0.34)	0.071 (0.27)	0.349 (0.59)	<0.001 (<0.001)
Population	0.852 (0.92)	0.373 (0.61)	<0.001 (<0.001)	<0.001 (<0.001)

The best model describing display behaviour was clearly superior to the possible alternatives with an AIC weight (w_i) of 0.723 indicating a high degree of support. The w_i for the best 'sneaky mating' model was 0.232 and there were four alternative models with ΔAIC values ≤2 **(Table 3)** indicating their position as acceptable alternatives (Burnham and Anderson 2001).

Following behaviour

The mixed effects model which best described the binomial component (initiation of the behaviour) had the same structure as the best display and 'sneak mating' models **(Table 2)**. For the non-zero, gamma distributed data (describing the duration of the behaviour) the best model included light (increasing light had a negative effect) and time period **(Table 2)**. Temperature had a significant effect with increasing temperature associated with a reduction at dawn and an increase at noon whereas light had a positive effect at dusk. There was one alternative model with an acceptable level of support (ΔAIC values ≤ 2).

When comparing between time periods, following behaviour did not differ significantly (Kruskal-Wallis χ^2= 3.3, df = 2, p-value = 0.191) although in both models the coefficient estimates for following behaviour were highest at dawn and much lower at dusk and noon **(Table 2, Figure 3)**. When males followed a female (durations greater than zero) there were significant differences between time periods (Kruskal-Wallis χ^2= 14.99, df = 2, p-value < 0.001) with the significant differences between dawn and noon (Kruskal-Wallis χ^2= 10.25, df = 1, p-value = 0.001) and dawn and dusk (Kruskal-Wallis χ^2= 12.38, df = 1, p-value < 0.001). Noon and dusk were not significantly different (p = 0.787).

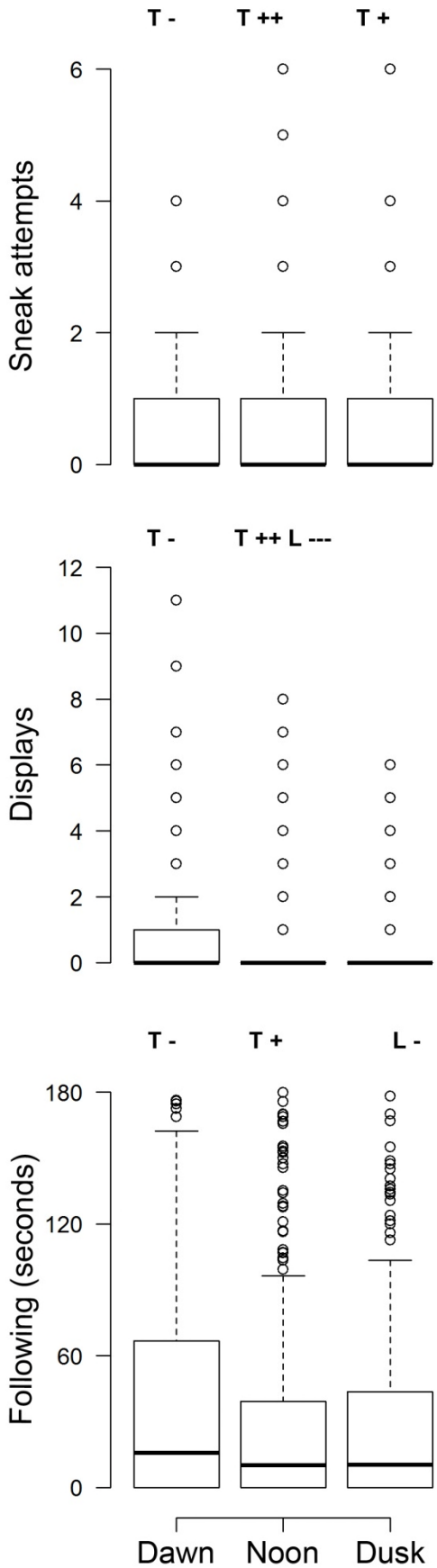


Figure 4: The effect of temperature and light on guppy mating behaviours recorded during three-minute observations at dawn, noon and dusk. Symbols represent the direction of the slope for the temperature and light coefficients (**+** = positive, **-** = negative). Number of symbols represents the significance level: **+/-** = 0.05; + +/– – = 0.01; + + +/– – – = 0.001.

Table 3: Multivariate models used to predict mating behaviours in guppies. AIC = Akaike Information Criterion, ΔAIC = AIC value – AIC of best model with values ≤ 2 indicate acceptable alternative models , w_i = AIC weight a measure of the relative likelihood of a model, given the data, ranging between 0 and 1; with AIC weights closer to 1 indicating high support for the model.

Model	AIC	ΔAIC	W_i
Display behaviour			
(Display~(log(Light)+Temp)*TimePeriod)	1132.045	0	0.723
(Display~log(Light)*Temp*TimePeriod)	1134.268	2.2	0.214
(Display~log(Light)*TimePeriod)	1137.092	5	0.063
(Display~((log(Light)*Temp)+TimePeriod))	1157.084	25	0
(Display~log(Light)+Temp)	1157.632	25.6	0
(Display~log(Light)*Temp)	1157.968	25.9	0
(Display~log(Light)+Temp+TimePeriod)	1158.316	26.3	0
(Display~log(Light))	1169.564	37.5	0
(Display~Temp)	1171.888	39.8	0
(Display~TimePeriod)	1172.845	40.8	0
(Display~1)	1198.24	66.2	0
'Sneak mating' behaviour			
(Sneak~(log(Light)+Temp)*TimePeriod)	885.129	0	0.232
(Sneak~log(Light)*Temp)	885.549	0.4	0.213
(Sneak~((log(Light)*Temp)+TimePeriod)	886.7866	1.7	0.11
(Sneak~Temp)	886.9546	1.8	0.109
(Sneak~log(Light)*TimePeriod)	887.1479	2	0.092
(Sneak~TimePeriod)	887.8817	2.8	0.068
(Sneak~1)	888.3075	3.2	0.056
(Sneak~log(Light)+Temp)	888.9498	3.8	0.04
(Sneak~log(Light)*Temp*TimePeriod)	888.9761	3.8	0.031
(Sneak~log(Light)+Temp+TimePeriod)	889.7393	4.6	0.026
(Sneak~log(Light))	890.0077	4.9	0.024
Following behaviour (gamma)			
(nz~(log(Light)+Temp)*TimePeriod)	946.076	0	0.498
(nz~log(Light)*Temp*TimePeriod)	946.194	0.1	0.423
(nz~Temp*TimePeriod)	951.218	5.1	0.041
(nz~log(Light)+Temp)	951.476	5.4	0.039
Following behaviour (binomial)			
(Follow~log(Light)*TimePeriod)	5483.02	0	0.599
(Follow~Temp*TimePeriod)	5485.16	2.1	0.205
(Follow~log(Light)+Temp)	5486.02	3	0.147
(Follow~(log(Light)+Temp)*TimePeriod)	5488.1	5.1	0.042
(Follow~log(Light)*Temp*TimePeriod)	5491.34	8.3	0.007

Discussion

In this experiment I was interested in how daily mating activity was affected by natural variability in environmental conditions. The results demonstrate that both light and temperature influence male guppy mating behaviour, and that they can have opposing effects depending on the time of day. No discernible pattern was observed for sneak mating attempts, but the pattern in display and following behaviour matched my prediction based on temperature (highest at dawn and lower at noon and dusk). However, mating activity continued to be recorded across time periods despite considerable variation in temperature and light representing a combined effect of abiotic conditions and the males' resilience to perform mating activity regardless of conditions. These patterns of activity are consistently seen in fish originating from different locations indicating that the results are robust.

Display and following time were lowest during the middle of the day which is in agreement with previous findings (e.g. Endler 1987, Archard et al. 2009). However, the results also suggest that mating activity is much higher at dawn than at dusk. At noon, display behaviours were least frequent yet the model shows that higher temperature causes an increase in display rate while higher light levels causes a decrease **(Figure 3)**. This effect of light is in keeping with other researchers' findings (e.g. Endler 1987, Reynolds et al. 1993, Long and Rosenqvist 1998) who demonstrated that high light intensity led to a reduction in number of displays, possibly as a result of increased perception of risk because of visibility to predators. Novel to this experiment however, is the observation that mating

behaviour remained low at dusk suggesting that either light level became too low for displays to be effective or that an effect outside of light (such as elevated temperatures continuing through the afternoon or because of alternative factors relating to the time of the day) led to the observed reduction. The effect of higher temperatures at noon causing an increase in display behaviour is more difficult to explain; (Muñoz et al. 2012) demonstrated a "physiological tipping point" for guppies exposed to 30^0C temperatures where metabolic demands have a negative effect on growth and locomotion. However, our data show that guppies continue to perform mating behaviours at temperatures in excess of these. I suggest that at elevated temperatures the fitness benefit (paternity) of opportunistic mating compensates for the cost of energy expenditure which may not be the case for functions that are less directly connected to fitness.

The number of 'sneak mating' attempts that males engage in is not obviously linked to abiotic conditions (e.g. Endler 1987, Reynolds et al. 1993) and appears to be better explained as an opportunistic tactic employed by males regardless of condition (although see Chapman et al. 2009). Accordingly my best model describing 'sneak mating' behaviour had a number of potential alternatives each with low AIC weights, and other factors such as female size (Magellan et al. 2005), competition (Ojanguren and Magurran 2004) and gonopodium length (Reynolds et al. 1993) could be better predictors.

Guppies are a highly successful invasive species and a contributing factor to this success may be a high degree of phenotypic plasticity (Torres-Dowdall et al. 2012a) which could have originated, in part, from the variable environmental

conditions found in the guppy's evolutionary history. However, some of the regions where guppies have invaded have very different daily and seasonal thermal and light regimes to those experienced in Trinidad (Deacon et al. 2011). At higher latitudes and altitudes temperature will become increasingly important in controlling daily activity and range expansion as conditions approach the lower limit of the guppy's thermal tolerance. For example, in the Mexican plateau, where guppies have become established (Lyons et al. 1995), there is a strong seasonal trend in temperature with winter temperatures far below those of the summer which are themselves at the lower end of temperatures experienced in Trinidad (Camacho-Cervantes *pers comm*).

In summary, this experiment has underlined the broad thermal tolerance of guppy mating activity. It demonstrates that the guppy's mating activity is modulated by temperature as well as light and that the effect of abiotic conditions can differ according to the time of day. A strength of my experimental approach is that I examined the behavioural responses to these twin drivers under natural conditions. However, as temperature and light covary in nature and my experimental design generates correlative results, environmentally controlled experiments are necessary to see if their effects are completely separable from each other as well as from time of day.

These results also show that water temperature in tropical freshwaters can change considerably over short temporal scales. Organisms which inhabit thermally variable environments tend to be generalists with broad temperature tolerance (Angilletta 2009). The ability to withstand sub-optimal conditions and

undertake foraging and reproduction during suitable periods will enable guppies to inhabit a wide range of thermal environments and may be the first step towards successful invasion and possible adaptation (Engel et al. 2011).

I suggest that this hitherto unappreciated variability in natural conditions has contributed to the resilience of male behaviour to the extent that males continue to pursue and court females throughout the day, and helps explain why the species has become a successful invader. My results highlight the need to consider the effects of temperature at different temporal scales and its interactive effect with light during ecological investigations. Furthermore, it is important to incorporate thermal variability into models predicting the effects of changing environmental conditions on species in order to get more accurate predictions (Arrighi et al. 2013). I shall, therefore, be exploring the life history consequences of thermal change using ecologically realistic thermal regimes in **Chapters 5 and 6**.

Chapter three: Thermal breadth of mating behaviour in the guppy

: guppies perform mating behaviours across a broader range of temperatures than those they experience in the wild… #ThermalGeneralists

Lay summary

Thermal performance curves (TPCs) are a visual way of exploring how temperature affects different aspects of an organism's performance. From this information we can then determine the upper and lower thermal limits and optimum temperature for the observed performance trait. In this experiment I exposed guppies to a new temperature for a short period of time (<24 hours) and recorded the mating behaviour of male guppies. The results confirm the fact that guppies are capable of withstanding a wide (17^0C) range of temperature with mating behaviour recorded at test temperatures from 17 to 34^0C. The water temperatures I have recorded in Trinidad range between 23 and 34^0C and so the most intriguing part of my results is that male guppies continue to attempt mating at temperatures much lower than they experience in the wild.

Abstract

In order to make predictions about the effect of climate change on an organism it is necessary to first describe the fundamental effect of temperature on aspects of its fitness. Thermal performance curves (TPCs) are a powerful tool for describing an organism's relationship with temperature and in this experiment I investigate the effect of acute temperature change on male guppy mating behaviour. Guppies continued to demonstrate mating activity across the entire 17^0C test temperature range (from 17 to 34^0C). Linear modelling indicates that the optimal temperature for mating behaviours was about 24^0C for courtship displays and following behaviour but much higher at approximately 28^0C for sneak mating. These results demonstrate that guppies have a broad thermal tolerance for mating behaviours and what is particularly interesting is that the lower part of this range is well below the lowest temperatures guppies ever experience in Trinidad or in our aquarium.

Keywords

Acute temperature change – mating behaviours – thermal tolerance – optimum temperature

Introduction

Temperature has a direct effect on ectotherms by controlling metabolic rate which in turn mediates all aspects of physiology such as activity and allocation of energy (e.g. Steinhausen et al. 2008, Allen et al. 2012). Knowing the fitness effects of these physiological changes and what are the ecological consequences is an essential parameter requirement for predictive models projecting the effects of climatic change (see Dell et al. 2011, Somero 2011, Zeh et al. 2012).

Macro-ecological patterns can be used to describe ecological outcomes such as geographic range limits and also predict the outcome of changing environmental conditions. The difficulty lies, however, with linking effects at the individual level to ecosystem-wide outcomes (Kearney et al. 2010) with focus beginning to develop on trait-based ecology (Chown 2012). Incorporating behavioural, physiological and genetic data into evaluations of species' vulnerability is necessary to enable scientists and managers to identify the organisms and habitats most sensitive to environmental perturbation (Huey et al. 2012). Consequently trait-based approaches to conservation, which incorporates both physiology and ecology, are becoming increasingly employed to predict the effect of individual responses on multi-species assemblages (Chown 2012).

Thermal performance curves (TPCs) are a type of reaction norm which describes, as a mathematical function, how the performance of an individual changes over a continuous range of temperatures (Kingsolver et al. 2001). TPCs are a powerful way of determining the thermal niche of an organism by characterising the effect of temperature on performance traits. The TPC provides

several important parameters including the temperature at which a performance trait is maximised (T_{opt}) and the upper (CT_{max}) and lower (CT_{min}) temperatures where performance is zero (Schulte et al. 2011). With this knowledge TPCs can be used to make predictions about the consequences of environmental change at an individual level and how they scale up to ecosystem-wide effects (e.g. Angert et al. 2011, Huey and Kingsolver 2011).

A species' thermal tolerance is commonly correlated with their environment (e.g. Clusella-Trullas et al. 2011) and temperature plays an important role in defining an individual's geographic range through thermal tolerance as well as by limiting performance (Schulte et al. 2011). Generally, species in high latitudes have a broader thermal tolerance and lower thermal tolerance limits than species closer to the equator (Gaston et al. 2009, Sunday et al. 2011). Consequently, tropical ectotherms are, arguably, most at risk from climate change as they already inhabit a hot environment and metabolism increases exponentially with temperature (Dillon et al. 2010) making even small increases in temperature more likely to be damaging. In order to determine the severity of this risk it is therefore necessary to understand the effect of temperature of important performance traits that can act as proxies for an individual's fitness.

Freshwater systems are suffering from a disproportionally high rate of biodiversity loss not least because they support such a large volume of biodiversity in a small area of the Earth's surface (Dudgeon et al. 2006). These losses are brought about by a number of human imposed factors and on a global- to local-scale, environmental temperature change is commonly ranked as the primary

driver (see Heino et al. 2009 for review). Freshwater fish can naturally experience rapid or short term temperature changes through vertical migration, daily variation in cloud cover or through increased water influx from high rainfall. However the increasing occurrences and outcomes from extreme weather events offer a new source of acute exposure to challenge species.

Guppies have been demonstrated to be a resilient fish with a flexible phenotype. This is cited as being responsible for their ubiquitous success as an invasive species whereby they have established populations in over 70 countries worldwide (Magurran 2005, Deacon et al. 2011). Despite invading areas with very different thermal environments to their native range guppies have successfully established and out-competed native assemblages. Limited seasonal temperature variation in low latitudes is cited as the general rule which leads to tropical species having narrower thermal tolerance ranges than organisms from higher latitudes (Sunday et al. 2011). However, the thermal heterogeneity experienced by guppies in Trinidad on a daily basis is greater than previously considered and may have important biological consequences. Recently collected water temperature data from Trinidad has shown that temperatures vary over the course of the day by as much as 10^0C with little seasonal variation **(Chapter 2, Appendix 1)**. This daily variability of water temperature represents an underestimated source of variation and is potentially key to the guppy's success as an invader by acting as selective pressure causing a broad thermal tolerance (Folguera et al. 2009) and the evolution of generalism (Ketola et al. 2013).

In this experiment I wanted to investigate the breadth of the guppy's thermal tolerance using mating behaviour as my performance measurement and address the question:

What are the immediate effects of temperature change on mating behaviours?

The specific aim of the experiment was to establish a TPC for male guppy mating activity. The TPC will enable estimates of the total performance breadth, optimal performance range (P_{opt}) upper (CT_{max}) and lower (CT_{min}) limits and thermal optimum (T_{opt}) over which the guppies perform different mating behaviours.

I chose to investigate whole body measures of fitness, in the form of mating behaviours, in order to link the effect of temperature with ecologically relevant consequences (Huey and Stevenson 1979). In the same way that physiological parameters can be used to measure the extent of environmental impacts on fishes (e.g. Vanlandeghem et al. 2010) whole body measures – such as mating behaviour counts – have the potential to be non-fatal measures of environmental stress (e.g. Wilson 2005) and can be applied to predicting ecosystem-wide changes (Chown 2012).

I predicted that male guppies will exhibit mating behaviours across a broad range of temperatures similar to those experienced in their native environment (24 – 32^0C). Following predictions based on the *beneficial acclimation* hypothesis (Leroi et al. 1994) I expected a peak in mating behaviour at temperatures close to those at which they have been housed in our aquarium for recent generations

(~24^{0}C). The alternative to this is the *warmer is better* hypothesis where performance will increase with increasing metabolic rate until the point at which temperatures become detrimental and performance rapidly tails off (Huey and Kingsolver 1989).

Describing the guppy's TPC enables us to understand the breadth of an organism's thermal tolerance. With this knowledge it becomes possible to identify how it changes when exposed to novel conditions and make predictions based on forecasted changes in environmental temperature.

Methods

The guppies used in this experiment were descendants of guppies originally collected from a lower portion of the Tacarigua River in Trinidad that have been housed for several generations in the aquarium at St Andrews. Temperature within the aquarium averages around 24.3^{0}C (sd = 1. 43, number of measures = 8708) with some variability between tanks **(Figure 1).**

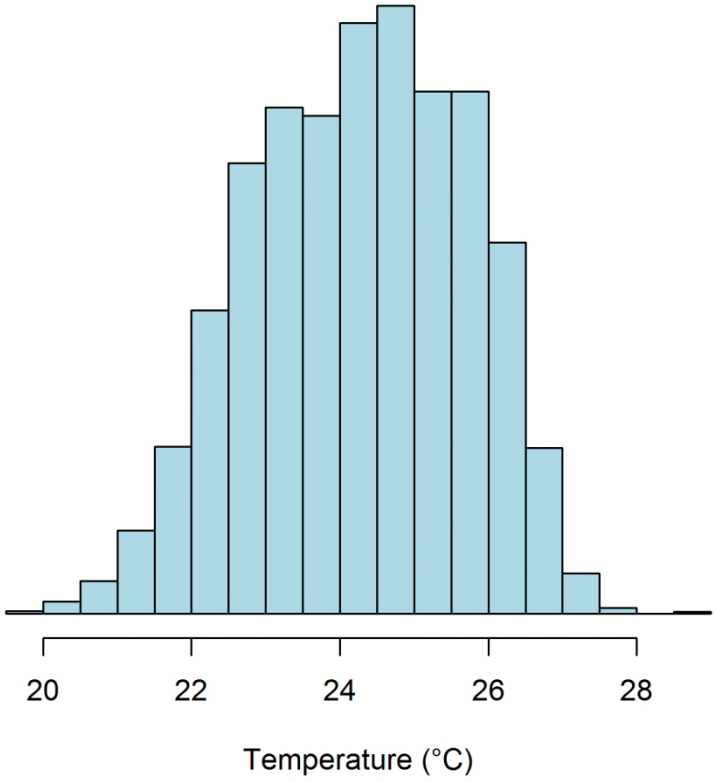


Figure 1: Histogram of the temperatures experienced by guppies in the St Andrews aquarium.

Two 40 litre aquarium tanks to be used for behavioural observations were filled with aged tap water and aerated using an air stone. Gravel was added to the bottom of the tank and a 150 w aquarium heater (Deltatherm, Interpet Ltd.) provided the temperature control. A further 40 litre tank was set up with a recirculating temperature control system to act as a water bath.

The day before each trial, experimental fish were placed in a petri dish filled with aquarium water alongside a measurement scale and photographed from above. The standard length of each fish (sl) was then determined from these images using the *ImageJ* image processing program (http://imagej.nih.gov/ij/). The fish were then placed within individual containers into the water bath where the water temperature was then increased or decreased to a target temperature at no more than 2^0C per hour).

On the day of the trial, three male and two female fish were added to an observation tank, where the water temperature had been heated/cooled to match that of the water bath, and allowed to settle for 20 minutes. Multiple fish were used to minimise individual stress and reduce the intensity of attention received by the female fish. To ensure that familiarity did not suppress motivation for mating (Hughes et al. 1999, Kelley et al. 1999), male and female fish were picked from separate stock tanks where they had been kept apart for over one month.

Each male was observed for 10 minutes and their mating behaviours recorded using the *JWatcher* behavioural scoring software (www.jwatcher.ucla.edu). The behaviours recorded were: the numbers of *sigmoid displays* and non-consensual *sneak mating* attempts and the total time spent *following* a female (a measure of mating intent). After all three of the males had been observed the heater was turned off and water left to return to the ambient temperature before fish were replaced into stock tanks

To minimise the effect of female size biasing the amount of mating behaviours, focal females were each re-used three times with fish randomly

assorted between treatments. In total 28 trials were run each with three males individually observed leading to 84 observation events at temperatures between 17 and 34^0C. Average (± sd, n) sl was 15.8 mm (± 0.1, 84) for males and 20.9 mm (± 0.2, 56) for females.

Analysis

The data were analysed using the generalised linear model (glm) function in R with *temperature*, *male size* and an *average size of the two females* used in each trial (plus interactions between variables) as the potential independent variables. A Poisson error distribution was assumed for the discrete dependent variables (number of *displays* and *sneak mating* attempts) and a gamma distribution for the continuous (time spent *following*) variable. The relationship between performance traits and temperature is best described by an asymmetric curve. With increasing temperature, performance increases to a performance maximum (P_{max}) before rapidly decreasing at temperatures over the thermal optimum (T_{opt}) (Huey and Stevenson 1979, Schulte et al. 2011). In order to capture the shape of this non-linear relationship, therefore, temperature was included in the models as a *linear*, *quadratic* and *cubic* polynomial to determine which explained the most variance. A cubic polynomial would represent a sharper decrease past the T_{opt} than the quadratic and therefore a smaller increase in temperature would result in a faster loss of performance. The T_{opt} was determined by solving the polynomial function for when the first derivative equals zero and the optimal performance breadth (P_{opt}) is the temperature range where behaviours are at least 80% of the P_{max} (Angilletta et al. 2002, Schulte et al. 2011).

Model selection was performed by minimising Aikaike's information criterion (AIC; Akaike 1973) values. AICc, a derivative of AIC used for small sample sizes (Johnson and Omland 2004), was used to compare time spent following. As count data is commonly overdispersed the Quasi-AIC (QAIC) value was employed for comparing the display and sneak mating models as it takes overdispersion into account (Burnham and Anderson, 2001, Richards 2008). The AIC weights (wi) are reported as a measure of the relative likelihood of each model, within those compared, ranging between 0 and 1 with weights closer to 1 indicating higher support (Johnson and Omland 2004).The D^2 value, which is the glm equivalent of R^2, provides a value of the deviance explained by each model. D^2 was calculated using **Equation 1** where the null deviance is the deviance of the intercept-only model and the residual deviance is the remaining unexplained deviance after all the variables have been included (Guisan and Zimmermann 2000). The better the fit of a model the lower the residual deviance and the closer D^2 is to 1.

Equation 1:

$$\boldsymbol{D^2 = (null\ deviance - residual\ deviance)\ /\ null\ deviance}$$

The adjusted D^2 (analogous to the adjusted R^2) takes into account the number of observations (n) and predictors (p) providing, arguably, a more representative measure of the model's fit **(Equation 2)** (Guisan and Zimmermann 2000). The adjusted D^2 can therefore be used to compare models with different variables and interactions.

Equation 2:

$$\boldsymbol{Adjusted\ D^2 = 1 - [(n-1)\ /\ (n-p)] \times [1 - D^2]}$$

Results

Male mating behaviours were recorded across all test temperatures (17 – 34^{0}C) demonstrating that male guppies were reproductively active across a temperature range of 17^{0}C. There was also a great deal of variability between individuals resulting in a poorly explained relationship between temperature and mating behaviour **(Figure 2)**.

The model which best described the observed pattern of sigmoid displays included temperature squared (second order polynomial) along with male size. As temperature increased to a T_{opt} of 24.1^{0}C guppies are increasingly likely to initiate display behaviour before a decline past this point as fewer guppies chose to perform displays. Increasing male size was also correlated with an increase in the numbers of displays.

The choice of best model for time spent following was simpler with temperature squared the only retained coefficient and similar to display behaviour the T_{opt} was 23.63^{0}C. For sneak mating attempts the T_{opt} was 28.26^{0}C with temperature squared and cubed as the explanatory variables in the best model. There was, however, very little to distinguish between the models including temperature squared and temperature cubed with the cubic model favoured by just 0.11 QAICc points and explaining only 1.5% more deviance.

The optimal performance breadth (P_{opt}), where behavioural traits are within 20% of the P_{max} (Angilletta et al. 2002), was similar for display (19.4 – 28.9^{0}C) and following (19.1 – 28.2^{0}C) behaviour and much narrower (24.6 – 31.2^{0}C) for sneak mating **(Table 1)**.

Table 1: Parameters present in the best models describing the observed data for each mating behaviour; % deviance explained (adjusted D^2); T_{opt}, where the derivative of the polynomial curve is zero; and P_{opt}, which is the range of temperatures where performance was within 20% of the P_{max}.

Behaviour	**Parameters in best model**	**Adj. D^2**	**T_{opt} (^{0}C)**	**P_{opt} (^{0}C)**
Display	Temp, temp^2, male size	7.1	24.14	19.39 – 28.89
Sneak mating	Temp, temp^2, temp^3	12.5	28.26	24.65 – 31.16
Following	Temp, temp^2	9.4	23.63	19.08 – 28.17

The (Q)AIC weight of the models selected for both following and display behaviour were clearly better than the rest of the candidate set of models whereas for the sneak mating data there were multiple similarly weighted models **(Table 2)**. For all three behaviours however, there were a number of models within 7 (Q)AICc values to the selected model and this combined with the relatively low (Q)AICc$_{wi}$ indicates that model selection uncertainly remains (Symonds and Moussalli 2010).

Table 2: (Q)AICc and adjusted D^2 values used in model selection for each mating behaviour. The lower the (Q)AICc value the better the model. Adjusted D^2 gives a value for the deviance explained by each model, the difference in D^2 values between models can be used to estimate the % deviance explained by individual parameters. (Q)AICc_{wi} gives a measure of the relative weight of evidence for each model out of the candidate set of models.

	Displays			**Sneaks**			**Following**		
Model parameters	QAICc	QAICc_{wi}	Adj D^2	QAICc	QAICc_{wi}	Adj D^2	AICc	AICc_{wi}	Adj D^2
Temp	304.98	0.000	0.010	143.94	0.006	0.009	982.54	0.011	0.024
Temp+ $Temp^2$	292.36	0.078	0.060	135.95	0.300	0.110	**975.45**	**0.367**	**0.094**
Temp+ $Temp^2$ + $Temp^3$	293.91	0.036	0.052	**135.84**	**0.317**	**0.125**	977.16	0.156	0.087
$Temp^2$ + Male size * Female size	292.90	0.060	0.052	142.21	0.013	0.084	980.69	0.027	0.074
$Temp^2$ + Male size + Female size	290.58	0.190	0.064	139.83	0.043	0.095	978.38	0.085	0.085
$Temp^2$ + Male size	**289.35**	**0.353**	**0.071**	137.54	0.135	0.106	976.51	0.216	0.093
$Temp^3$ + Male size * Female size	294.28	0.030	0.045	142.20	0.013	0.100	982.50	0.011	0.068
$Temp^3$ + Male size + Female size	291.95	0.096	0.057	139.76	0.045	0.112	980.10	0.036	0.079
$Temp^3$ + Male size	290.98	0.156	0.062	137.66	0.128	0.120	978.22	0.092	0.087

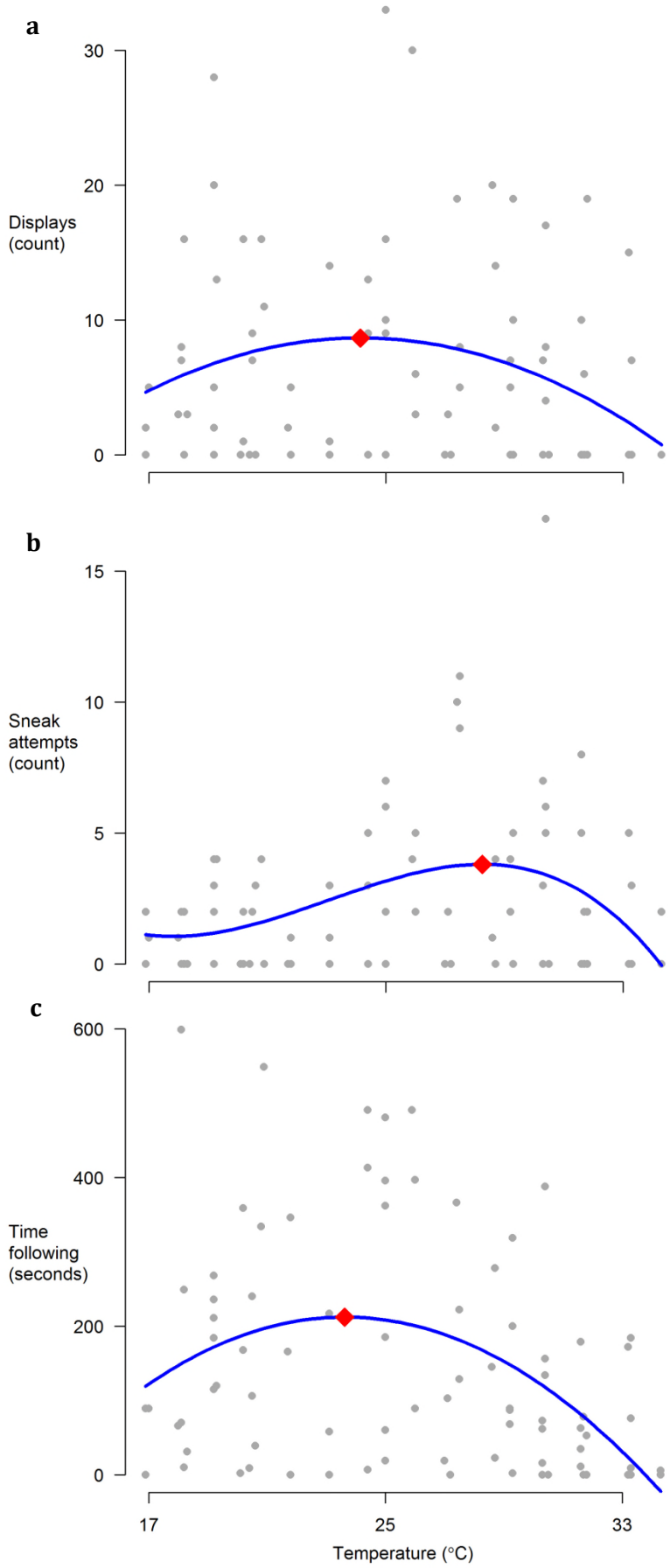


Figure 2: Number of displays **(a)**, sneaks **(b)** and following time **(c)** by male guppies at a range of temperatures. Blue line is polynomial curve describing the relationship between mating behaviour and temperature. Red diamond indicates temperature where mating behaviour is highest (T_{opt}).

Discussion

These results show that guppies continue to exhibit mating behaviours when exposed to a 17^0C range of temperatures between 17 – 34^0C. This range is much broader than that which these fish have experienced in the St Andrews aquarium for multiple generations or in their ancestral environment. There was a great deal of variability in mating behaviour across the entire range of test temperatures with some fish performing no mating behaviours and some performing multiple times at most of the temperatures. Modelling of the response indicates that temperature was an important predictor of mating behaviours however the proportion of variation explained by temperature was relatively low suggesting that other factors are necessary to explain the pattern better.

In this experiment there was a similar T_{opt} for display and following behaviour at ~24^0C which supports the *beneficial acclimation hypothesis* prediction. The T_{opt} for sneak mating, however, was more frequent in water ~4^0C warmer and this is more representative of the *warmer is better* hypothesis as warmer temperatures will facilitate energetic behaviours.

The pattern in display behaviours was explained as a combination of temperature as well as male size. Male size, however, accounted for only a small increase in the % deviance of just 1.1% indicating that temperature is responsible for the majority of explained variance **(Table 2)**. Size has previously been reported to affect display behaviour whereby larger males display more frequently (e.g. Abrahams 1993) and larger females attract more displays (e.g. Dosen and Montgomerie 2004) although there is contrasting evidence (Endler and Houde

1995, Ojanguren and Magurran 2004) suggesting size is not always a good predictor of mating behaviour.

For the other behavioural traits, including male or female size did not significantly improve the models. Previous work has indicated that sneak mating is more frequent in smaller fish (Bisazza and Pilastro 1997, Pilastro et al. 1997) however that was not apparent in this experiment and it is possible that the range of fish sizes was too small to distinguish any effect of size from temperature. Coercive (sneak) mating behaviour is dependent on swimming performance and agility (Wilson 2005) and this may explain the narrower performance range of sneak mating compared to the display and following behaviour. Burst swimming, a requirement for sneak mating, is more closely affected by acute temperature changes as burst swimming is closely associated with aerobic metabolic rate (Svendsen et al. 2013) which in turn is closely linked with temperature (Angilletta 2009). Consequently, a potential negative outcome for female fish at temperatures within the sneak mating performance range is that they will experience greater harassment from male fish. The effect of this could be to reduce foraging time or predator inspection behaviour which has obvious ecological implications (Magurran and Seghers 1994).

Although temperature (and fish size) explained a part of the observed data only a very small portion of the total deviance was explained. It is possible, therefore that my experimental temperature range was too narrow and was unable to capture the upper and lower bounds of the guppy's TPC. Similarly, the natural variability of individuals may have masked underlying patterns and so a

repeated measures design where individual guppies are repeatedly tested at a range of temperatures may have been a more powerful way of determining the effect of temperature.

The temperatures recorded in Trinidad indicate that guppies inhabit a thermally heterogenous environment. At downstream locations in Trinidad, water temperatures routinely reach >30^{0}C with temperatures varying by as much as 10^{0}C per day. However, in a year's worth of data collection I have not recorded temperatures lower than 23^{0}C even in colder upstream locations **(See Appendix 1)**. I have already demonstrated in **Chapter 2** that guppies are capable of responding to natural temperature variation behaviourally by altering mating behaviour however guppies must have also evolved a physiological capacity to withstand such variability and the ecological consequences of the guppy's ability to tolerate novel environments is well documented (Deacon et al. 2011). It is apparent that even in low latitudes daily variation in temperature can be quite considerable and should not be overlooked as a source of variability driving behavioural, physiological and geographical patterns (e.g. Arrighi et al. 2013, Paaijmans et al. 2013, Reeve et al. 2014). The 17^{0}C range of temperatures over which guppies continue to perform mating behaviour is an example of the phenotypic flexibility which has enabled guppies to become such a resilient species and successful invader. Theoretical predictions suggest that environmental heterogeneity is responsible for developing a broader thermal tolerance range (Stevens 1989, Ketola et al. 2013) however the evolution of the guppy's low temperature thermal tolerance is surprising as water temperatures below 20^{0}C are unlikely to be routinely encountered in their native environment.

Guppies have successfully established in areas where water temperatures exhibit strong seasonal variation and fall much lower than those of Trinidad. It is therefore evident that guppies have a natural ability to tolerate a range of temperatures broader than those experienced in Trinidad. In this experiment I successfully used a whole body measure as a non-invasive way of investigating the effect of temperature on an important aspect of fitness. Short term temperature exposure, however, provides a somewhat artificial snapshot of a species' ability to function at a certain temperature. Although mating behaviours were recorded across the temperature range there was no measure of their success, nor their 'cost' to males as the two tactics require different amounts of energy (Houde 1997) and transfer different volumes of sperm (Pilastro and Bisazza 1999). Furthermore, as exposure to elevated temperatures is metabolically costly the resultant oxidative stress may have long term fitness or lifespan implications not captured by our experimental approach (Dillon et al. 2010).

This experiment does, however, provide an indication of the thermal tolerance breadth of the guppy's mating behaviour which is comparable to that of wild guppies in Trinidad. In **Chapter 2** I recorded mating behaviour of wild caught guppies over successive days in experimental tanks across a 9^0C (23.4 - 32.4^{0}C) range. There was cyclical pattern entrained to light and temperature, however, as behaviours were more frequently recorded during the early hours of the day when light was less intense and temperatures were between 24-25^{0}C **(see Chapter 2)**. Using our understanding of the guppy's thermal tolerance gained from this experiment I can now investigate the effects of prolonged thermal exposure and the guppy's ability to acclimate to some of these temperatures **(see Chapter 4)**.

Chapter four: Thermal regime change leads to incomplete acclimation of life history traits

: guppies respond to thermal regime change in their mating behaviour & by altering offspring size & growth… #TheAdaptableGuppy

Lay summary

Acclimation is the ability of organisms to adjust their performance so that it is optimal at the conditions in which they have recently been exposed. It is a way in which they can respond to changing environmental conditions such seasonal variation. As a general rule, organisms with a high degree of phenotypic flexibility ("adaptability") are also more capable of acclimating to different conditions. In this experiment I looked at the ability of guppies to adjust to novel conditions and what effect this adjustment had on their offspring. I found that guppies can acclimate successfully to a different thermal regime although not to the highest temperature treatment (despite them routinely encountering it in the wild). Offspring in the hotter treatment were smaller but grew faster than those in the colder treatments and this was modified by both environmental temperature and the temperature in which they developed as embryos. Modulating growth and body size will have implications for the guppy's fitness resulting in possible changes in fecundity, predator avoidance ability or lifespan. The ability to quickly adjust to different temperatures is an essential first step to successfully establishing in new environments.

Abstract

Understanding the phenotypic responses by species to a prolonged change in their thermal regime is essential for predicting the ecological consequences of environmental change. Acclimation can take place over a short timescale (weeks) and enable an organism to maintain performance levels in novel conditions although the breadth of conditions which can be acclimated to depends on the scope of the individual's phenotypic flexibility. We investigated the effect of thermal acclimation to three stable temperatures (24, 27 and 30°C) on guppy swimming activity, mating behaviour and on the size and growth of subsequent offspring. The 30°C treatment proved too stressful despite being a routinely encountered temperature in the wild. Mating activity was highest at an individual's acclimation temperature although it was highly variable and modelling failed to produce a clear result for the behaviour or activity responses we measured. Offspring size decreased at higher temperatures but there was no change in fecundity. Body size and growth followed theoretical predictions – larger and slower at lower temperatures – although both were affected by a combination of acclimation and environmental temperature. I conclude that when faced with novel conditions guppies are capable of adjusting life history parameters to the new conditions. However, prolonged exposure to stable temperatures at the upper range of their thermal niche, are ultimately detrimental and guppies are incapable of withstanding them.

Keywords

Thermal acclimation – growth – offspring characteristics – mating behaviour – tropical freshwaters – developmental effects

Introduction

Environmental temperature constrains latitudinal and altitudinal distribution and is one of the main barriers preventing range expansion (Gaston et al. 2009). The prospect of a changing climate, however, increases the potential for thermally flexible species to move into new environments and has resulted in a need to predict the likely consequences (Petitpierre et al. 2012). Tropical temperatures are less variable than those at higher latitudes and organisms are predicted to have narrower thermal tolerance ranges in these areas (Tewksbury et al. 2008, Sunday et al. 2011). Furthermore, as temperatures in the tropics are higher, tropical species inhabit temperature regimes that are already near to their thermal safety margin which reduces the scope of their ability to withstand increasing temperatures (Paaijmans et al. 2013, Sunday et al. 2014). Consequently, species which live in the tropics are more susceptible to increasing climate as they are closer to their thermal tolerance limits than temperate species (Stillman 2003, Deutsch et al. 2008).

Thermal acclimation is a phenotypic physiological response which takes place to compensate for the impact of environmental change (Huey et al. 1999). Many ectotherms naturally display regular changes in phenotype, generally through alterations in tissue structure and protein expression as a result of long term (usually seasonal) temperature changes (Wilson and Johnston 2004) and those species which experience regular or seasonal temperature variations are commonly those with the greatest capacity for phenotypic acclimation (reviewed in Johnston and Temple 2002). An organism's thermal tolerance and ability to

acclimate to changing conditions are critical aspects of its physiological niche and enables them to inhabit a thermal range which is broader than the range of temperatures they routinely experience (Gaston and Spicer 2001, Calosi et al. 2010).

Acclimation is a (generally) reversible form of phenotypic plasticity and allows an organism's performance optimum to change with the prevailing conditions. The thermal regime experienced during embryonic development, however, causes permanent phenotypic effects through a cascade of biochemical reactions that 'fixes' phenotypes (Wilson and Franklin 2002) and has lasting fitness consequences by altering morphology, growth and performance. Developmental temperature has irreversible effects on muscle thereby fixing the potential final size of an individual (e.g. Garcia de la serrana et al. 2012) and biochemical processes have their own specific temperature coefficients causing environmental temperature to affect cell differentiation and growth differently (van der Have and de Jong 1996).

Embryos are particularly sensitive to temperature and the thermal tolerance range of embryos is generally narrower than that of the adult and situated near to the optimal performance temperature of the adult (van der Have 2002). With increasing temperature both development time and offspring size at birth tend to decrease (reviewed in Gaston et al. 2009, Bownds et al. 2010). Longer development time tends to result in larger, more mobile and more competitive offspring but can also have negative fitness consequences by limiting the time for

active mating in seasonal environments or extending the time between successive generations (see Dmitriew 2011).

The size and number of offspring produced by a female represents an energetic investment decision based on the environmental conditions experienced by the mother and the environment the offspring are likely to encounter (Mousseau and Fox 1998). In relatively benign environments optimality theory predicts that offspring will be smaller and more numerous (Allen et al. 2008). As the environment conditions becomes adverse, increases in pollution (Hendrickx et al. 2003) or competition (Sibly et al. 1988, Marshall et al. 2006) will lead to fewer, larger offspring.

Temperature experienced both as an embryo and during growth has a powerful influence on ectotherm body size. The *temperature-size* rule states that higher temperature causes faster growth, earlier maturation and smaller size at maturity (Atkinson 1995). The fitness consequences of this general rule can be grouped into two broad, yet apparently contradictory, patterns; *bigger is better* and *hotter is better* (Kingsolver and Huey 2008). Being larger has multiple benefits based on the idea that larger organisms having competitive advantages over their rivals and are more capable of escaping predation (e.g. Smith and Fretwell 1974, Barbosa and Magurran 2010, Brandner et al. 2013). A higher optimal temperature, however, is linked with increased performance and higher maximum growth rates although with a corresponding smaller size at birth and final adult size (e.g. Knies et al. 2009, Angilletta et al. 2010).

My earlier work has shown guppies to live in a thermally variable environment **(see Chapters 2 and 3)** which has resulted in a broad thermal tolerance range. Like many freshwater ectotherms, however, they are restricted in their capacity to migrate or behaviourally thermoregulate and so when faced with changing environmental conditions they will need to first tolerate and second, if possible, adapt to the new conditions. I am interested therefore, in the question:

What are the immediate phenotypic changes in life history responses made by guppies when faced with an extended change in environmental conditions?

The aim of this investigation was to determine the ability of guppies to thermally acclimate at different stages of ontogeny. I created three temperature regimes based on the range of temperatures guppies experience in the wild and recorded the effect on male mating behaviours as well as male and female swimming activity. The size and growth characteristics of offspring born into each regime were measured and compared in order to separate the effects of developmental and environmental temperature.

Guppies demonstrate a wide range of plasticity in life history and behaviour and I have already shown that they inhabit a thermally variable environment. I therefore predicted thermal acclimation to have a positive effect on mating activity with guppies attempting to mate more frequently when tested at the temperature in which they were acclimated. In Trinidad, guppies from upstream locations are larger than the guppies found in downstream populations. This is commonly explained as a result of the presence of predators on the downstream populations, which are absent from upstream, resulting in smaller adult sizes. However water

temperatures in the downstream locations are warmer than upstream and the *temperature-size* rule would provide an alternative (though not necessarily competing) explanation for the observed pattern. Following the theory, I expected offspring characteristics to follow the *temperature-size* rule, with higher temperatures leading to smaller offspring size at birth, higher fecundity and faster growth.

If we are to understand the fitness implications and ecological consequences of changing environmental conditions we need to describe the thermal niche and identify resulting life history trade-offs made in response (Williams et al. 2008, Huey et al. 2012). This experiment aims to address some of these requirements.

Methods

Experimental animals were descendants of fish originally collected from the Lower Tacarigua River in Trinidad that have been housed in the aquarium in St Andrews for several generations at around 24^0C (mean temp range = 23.3^0C – 24.8^0C, sd range = 1.2-1.4, number of measures = 897-930, across 8 different tanks within the aquarium). Throughout the experiment, fish were fed daily to satiation using commercially available flake food.

Acclimation

Twelve (40 litre) acclimation tanks filled with aged tap water were heated to 24, 27 or 30^0C using individual aquarium heaters (Deltatherm 300w, Interpet Ltd.) giving four replicates per treatment and arranged in a randomised split plot design in the aquarium. Each tank was aerated by a bio-mechanical filter and gravel and a few stones were added as tank structure. Temperature data loggers (MicroLite lite5016, Fourier Systems) were placed in 5 of the tanks to record the water temperature every 30 minutes **(Table 1)** and the water temperature of each tank was checked daily using a digital thermometer**.**

Table 1: Temperature variability in five of the acclimation tanks. Temperature was recorded every 30 minutes during the acclimation period. Due to datalogger malfunction the 27^0C data is a representative value taken from a tank in the same location with the same heater but at a later date.

	24^0C	**24^0C**	**27^0C**	**30^0C**	**30^0C**
Mean	23.5	24.0	27.2	30.4	29.7
sd	0.12	0.39	0.21	0.21	0.08
Range	1.44	1.65	1.31	1.43	0.45
Count	2140	2140	3262	2140	2140

Mature guppies were taken from stock tanks, photographed and sorted according to size to ensure that the standard length of fish did not differ significantly between acclimation treatments (females $F_{2, 57}$, p-value =0.3; males $F_{2, 57}$, p-value = 0.6). Measurements of fish were made by photographing individuals alongside a scale and measured using the image manipulation software *ImageJ* (www.imagej.nih.gov/ij/). Five male and five females were placed into each of the acclimation tanks and left to acclimate for six weeks (considered long enough for acclimation to take place in poeciliids, (see Wilson et al. 2007b)) before being used in the behaviour trials. Individual tanks were inspected daily for juveniles and mortality. Deaths were recorded and any offspring observed were removed, photographed, measured and housed in separate juvenile tanks.

Mating behaviour

Six (15 litre) test tanks each with three masked sides were placed in a separate observation room and filled with aged tap water. Six 150w aquarium heaters (Deltatherm, Interpet Ltd.) were pre-set to temperatures between 23 – 33^{0}C and could thus be used interchangeably across the tanks to avoid tank effects. Each tank contained a bio-mechanical filter which was removed immediately before each observation.

Twenty four hours prior to each test, a male and a female from the same acclimation temperature – but different acclimation tanks – were placed within separate containers into a water bath. The water temperature was then raised or lowered to the appropriate test temperature at no more than 2^{0}C per hour. The fish were then added to the pre heated/cooled observation tank and kept apart

until 15 minutes before the start of the observations when they were released and allowed to settle. Observations lasted for 10 minutes during which time the number of sigmoid mating *displays* and *sneak mating* attempts (Houde 1997) as well as the time the male spent *following* the female (an indication of male reproductive motivation) were recorded using *JWatcher* (www.jwatcher.ucla.edu, **see Chapters 2 & 3** for more information about the behaviours). To capture the 3 dimensional movement and speed of each fish, observation tanks were recorded from the side and above – by way of a 45^0-angled mirror positioned above the tanks – using a Casio Exilim EX-FH25 video camera fixed to a stationary tripod **(Image 1)**. Once observations were completed, fish were re-housed in temperature controlled holding tanks which matched their acclimation temperature.

Juvenile characteristics:

Offspring number and size at birth

Any offspring observed in the acclimation tanks were immediately removed, counted, photographed for size measurement and either placed into a separate temperature controlled aquarium with water temperatures matching their birth tank temperature or used in the newborn growth experiment.

Newborn growth experiment

Offspring were placed individually into plastic 10 cm x 21 cm x 10 cm floating hatchery tanks that had small holes for water to circulate in and out (Marina, Hagen Inc.). These hatchery tanks were held in 40 litre aquarium tanks with the water maintained at a temperature between 18-33^0C for two weeks

during which time fish were fed daily **(Table 2)**. After this time, fish were removed, photographed and placed into a holding tank and the *specific growth rate* (SGR) of each fish determined.

Image 1: Tank, with 45^0-angled mirror, used for mating behaviour observations and filming. Filtration unit was removed before observations were made.

Table 2: Number of newborn growth replicates for offspring born into each acclimation treatment.

		Test temperature (^{0}C)					
		18	21	24	27	30	33
Acclimation temperature (^{0}C)	24	4	5	3	4	4	4
	27	3	4	3	4	4	4

Juvenile growth experiment

Twelve (15 litre) tanks were arranged in a split plot design in the aquarium with water temperature maintained at either 24^{0}C (grand mean =24.5 +/- 0.7 sd, range =22.7-26.4^{0}C) or 27^{0}C (grand mean = 26.9 +/- 0.8 sd, range = 25.3-30.7^{0}C). Up to seven offspring (24:24 mean = 0.82, range = 0.35, 24:27 mean = 0.76, range = 0.2, 27:27 mean = 0.82, range = 0.59, 27:24 mean = 0.77, range = 0.33) born within a two week period from each acclimation temperature were photographed and added to a treatment tank held at either their own acclimation temperature or the reciprocal one. This resulted in four treatments each replicated three times **(Table 3)**. Fish were fed to satiation daily using commercial flake food and photographed weekly for the duration of the experiment (11 weeks).

Table 3: The four thermal treatments (and codes) used in the juvenile growth experiment.

		Growth temperature (^{0}C)	
		24	27
Developmental Temperature (^{0}C)	24	Lower-Lower (LL)	Lower-Higher (LH)
	27	Higher-Lower (HL)	Higher-Higher (HH)

Analysis

The ten minute observation videos, filmed at 29.97 frames/second, were exported as a stack of images and reduced to one frame/5 seconds (120 frames). Using the *Manual Tracking* plugin for ImageJ, the coordinates of the length (X), width (Y) and depth (Z) of the tank within the frame are fixed. The position of the eye of each fish was then marked on every frame and the distance moved in three dimensions (X, Y and Z) between each frame was measured and summed using **Equation 1**.

Equation 1:

$$\sqrt{((\boldsymbol{X}-\boldsymbol{X_0})^2+(\boldsymbol{Y}-\boldsymbol{Y_0})^2+(\boldsymbol{Z}-\boldsymbol{Z_0})^2)}$$

Swimming speed for the distance moved in three directions between each frame was calculated and then averaged over the entire observation period. The *coefficient of variation* (CV) in swimming speed, which provides a measure of the variability of speed independent of the mean, was calculated by dividing the standard deviation of the swimming speed by the average swimming speed. Higher values indicate that a fish is swimming more erratically which is indicative of a stressed fish.

Specific growth rate (SGR) of newborn growth was calculated using the following formula **(Equation 2)**:

Equation 2:

$$\boldsymbol{G} = \big(\boldsymbol{Ln}(\boldsymbol{S_t}) - \boldsymbol{Ln}(\boldsymbol{S_i})\big)/\boldsymbol{t}$$

Where instantaneous growth (G) is a function of the natural logarithm of size at time *t* (S_t) minus the natural logarithm of the original size (S_i) divided by *t* the duration of the growth period (7 days) (Hopkins 1992).

Juvenile growth was modelled using a modified Von Bertalanffy growth model (Beverton 1954, Cailliet et al. 2006) **(Equation 3)**:

Equation 3:

$$\boldsymbol{L(t)} = \boldsymbol{L_\infty}\big(\mathbf{1}-\boldsymbol{e}^{-\boldsymbol{k}(\boldsymbol{t}-\boldsymbol{t_0})}\big)$$

Where *L(t)* is length as a function of time and *Linf* (L_∞) is the theoretical asymptotic length. *k* is the Brody growth rate coefficient (the rate at which a fish

approaches its asymptotic length) and t_0 the theoretical time at length zero. Von Bertalanffy parameters were calculated using the FSA package (Ogle 2012) in R. The growth data was bootstrapped 1000 times to create 95% confidence intervals for each of the parameters and make predictions up to 20 weeks.

It is inadvisable to use a MANOVA approach when the response variables are correlated, count data and from a small sample size, and so each variable was investigated independently. *Male* and *female distance travelled* and *CV* were log transformed, so that the data approached a normal distribution, and modelled using linear models. *Display* and *sneak* behaviours had a Poisson distribution and time spent *following* a gamma distribution and were consequently modelled using generalised linear models. Data were analysed using R, version 2.15.1 (R Core Team 2012). Model selection was made using a backward selection approach beginning with the full model and removing non-significant coefficients. The final model was selected by having the lowest (Q)AICc value **(see also Chapter 3)**.

Results

Acclimation:

During the acclimation period mortality was much higher in the 30^{0}C treatment than both the 27^{0}C (Z-Score = 3.005, p-value < 0.01) and 24^{0}C (Z-Score = 4.284, p-value < 0.01) treatments and was also significantly different between fish in the 24^{0}C and 27^{0}C treatments (Z-Score = 1.698, p-value < 0.05) **(Table 4)**.

Table 4: Sizes of fish placed into the tanks at the start of the acclimation period, also mortality and number of offspring born in each acclimation temperature.

	Female			**Male**		
Acclimation Temp. (^{0}C)	24	27	30	24	27	30
Average size	1.846	1.795	1.931	1.623	1.633	1.660
sd	0.22	0.26	0.33	0.12	0.12	0.11
Range	1.5-2.4	1.4-2.5	1.5-2.4	1.4-1.9	1.4-1.9	1.4-1.9
Mortality	0/20	3/20	11/20	1/20	2/20	6/20
No. offspring	95	87	25	-	-	-

Mating behaviour:

As a consequence of fish mortality during acclimation the number of behavioural trials was necessarily truncated. There were not enough fish that survived acclimation to 30^{0}C to replicate adequately and were therefore excluded from the behavioural trials. There was 17 trials performed by the 24^{0}C acclimated fish (temp range 22.7 - 33.2^{0}C) and 13 of the 27^{0}C acclimated fish (temp range 23 - 33.2^{0}C) although two trials were excluded because the fish behaved very erratically during the trials.

Linear regression indicated that there was no relationship between distance travelled or CV in swimming speed with temperature difference. For each of the mating behaviours the final model included the interaction between acclimation- and test temperature. Display behaviour also included male and female size as important coefficients as well as temperature as a second order polynomial ($D^2 = 0.61$); sneak behaviour was also best explained by treating test temperature as a second order polynomial but no other explanatory variables was retained in the best model ($D^2 = 0.12$); the final model describing following behaviour included only test temperature ($D^2 = 0.08$) **(Table 5)**. Plotting the behaviour counts/duration shows an increase in each of the mating behaviours when the difference between acclimation and test temperature is minimised, i.e. when the test temperature is closer to a fish's acclimation temperature **(Figure 1)**.

Table 5: Coefficient estimates of the 'best' models describing the guppy's mating behaviour, NA= not included in model, ns = present but not significant.

	Display			**Sneak**			**Following**		
	Estimate	Std.Error	Pr(>|z|)	Estimate	Std.Error	Pr(>|z|)	Estimate	Std.Error	Pr(>|z|)
(Intercept)	47.71	62.67	ns	46.56	23.56	< 0.05	-0.05	0.02	< 0.05
Acclimation Temp (27)	-213.04	74.75	< 0.01	-90.51	31.13	< 0.01	0.05	0.02	< 0.05
Test Temp,	-4.62	5.11	ns	-3.15	1.78	< 0.1	0.002	0.001	< 0.05
Test Temp^2	0.07	0.10	ns	0.05	0.03	ns	NA	NA	NA
Male Size	11.23	2.67	< 0.001	NA	NA	NA	NA	NA	NA
Female Size	2.95	1.06	< 0.01	NA	NA	NA	NA	NA	NA
AccTemp (27):TestTemp	15.26	5.94	< 0.05	6.44	2.33	< 0.01	-0.002	0.001	< 0.05
AccTemp (27):TestTemp^2	-0.27	0.12	< 0.05	-0.11	0.04	< 0.01	NA	NA	NA
D^2	0.61			0.12			0.08		

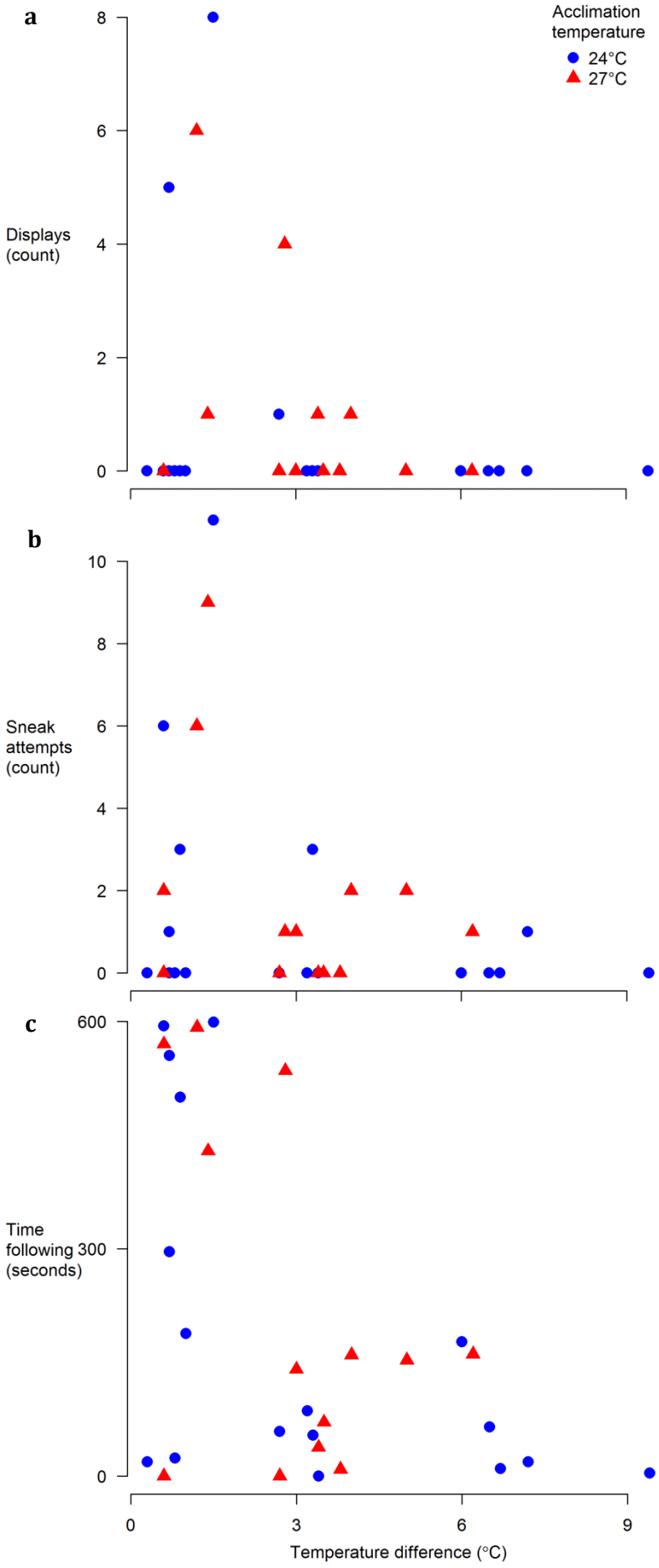


Figure 1: Plot of displays **(a)**, sneak attempts **(b)** and time spent following **(c)** against the temperature difference between acclimation and test temperature, circles= 24^0C, triangles = 27^0C.

Juvenile characteristics:

Offspring number and size at birth:

The total number of offspring born was similar for both the 24 and 27^{0}C fish (95 and 87) over the duration of the experiment (69 days) whereas in the 30^{0}C treatment, aside from a single offspring born on day 57, no offspring were born after day 36 **(Figure 2)**.Variability in offspring size was quite high (range (mm): (24^{0}C) = 2.6, (27^{0}C) = 2.4, (30^{0}C) = 2.7) but higher temperatures resulted in significantly smaller offspring size at birth ($F_{3,202}$ =11.08, p-value <0.01). Furthermore, as the acclimation period continued offspring born into the hotter temperatures became progressively smaller. An ANCOVA for offspring size versus acclimation temperature, controlling for exposure duration, demonstrated statistically significant effects of acclimation temperature ($F_{2,200}$ = 6.642, p-value <0.01), exposure duration ($F_{1,200}$ = 12.218, p-value <0.001) and the interaction between main effect and covariate ($F_{2,200}$ = 3.592, p-value <0.05). This indicates that temperature has an effect on offspring size over time and that the effect is different for each temperature. A regression of offspring size against time showed that offspring became progressively smaller over time in both the 27^{0}C (ANOVA, $F_{1,84}$ = 16, p-value <0.01) and 30^{0}C (ANOVA, $F_{1,23}$ = 3.679, p-value <0.1) treatments but not for the 24^{0}C treatment where offspring size did not significantly differ during the acclimation period (ANOVA, $F_{1,93}$ = 0.97, p-value = 0.33).

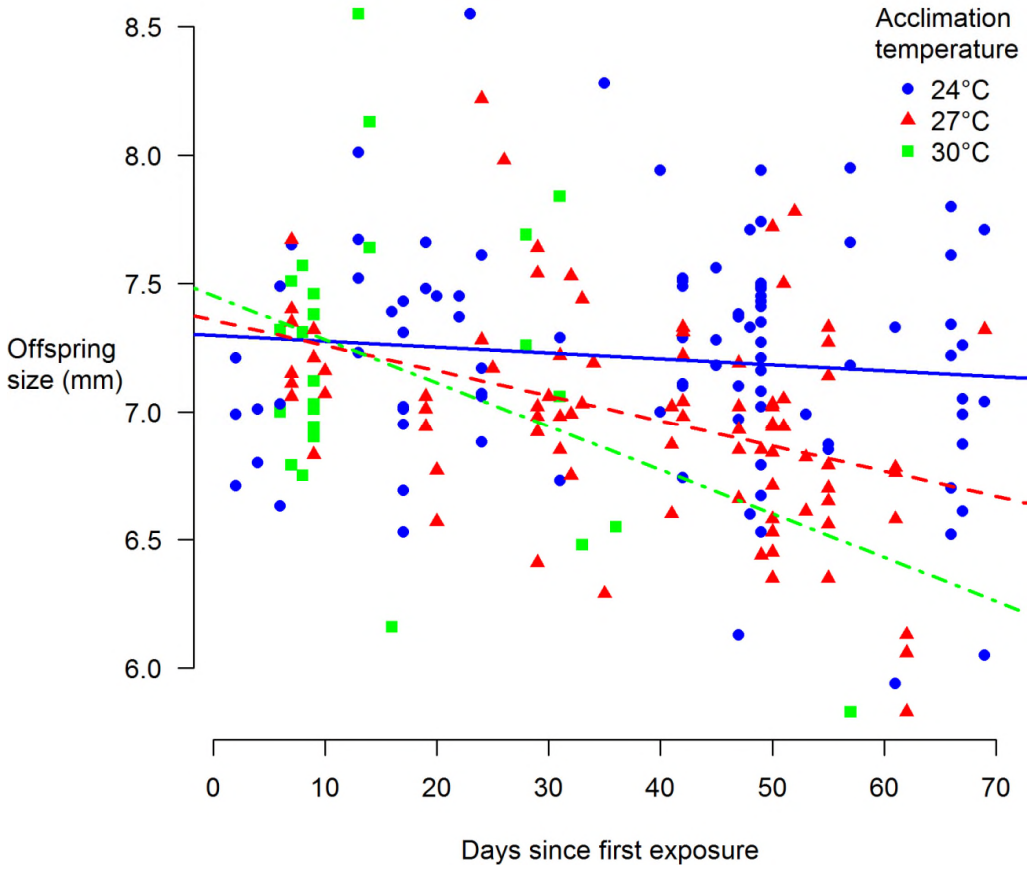


Figure 2: Offspring size of fish born into each acclimation temperature over exposure period (70 days). Blue circles = 24^{0}C, red triangles = 27^{0}C, green squares = 30^{0}C. Lines are the linear regression of offspring size against time (day). Solid line = 24^{0}C, dashed line = 27^{0}C, dot-dashed line = 30^{0}C.

Newborn growth:

Temperature as well as thermal history affected growth in newborn fish. Offspring born in the 24^{0}C treatment had a consistently higher SGR except at the highest test temperature (33^{0}C). Growth was fastest for both acclimation treatments at the test temperature of ~27^{0}C (24^{0}C = 26.8^{0}C, 27^{0}C = 27.5^{0}C) although it was nearly 40% faster for 24^{0}C acclimated fish at this point **(Figure 3)**. Both data series were fitted with third order polynomial curves which explained 61% ($y = -0.0015x^3 + 0.0907x^2 - 1.6473x + 9.7067$) and 43% ($y = -0.001x^3 + 0.0607x^2 - 1.1427x + 7.0601$) of the residual deviance for the 24^{0}C and the 27^{0}C treatments respectively.

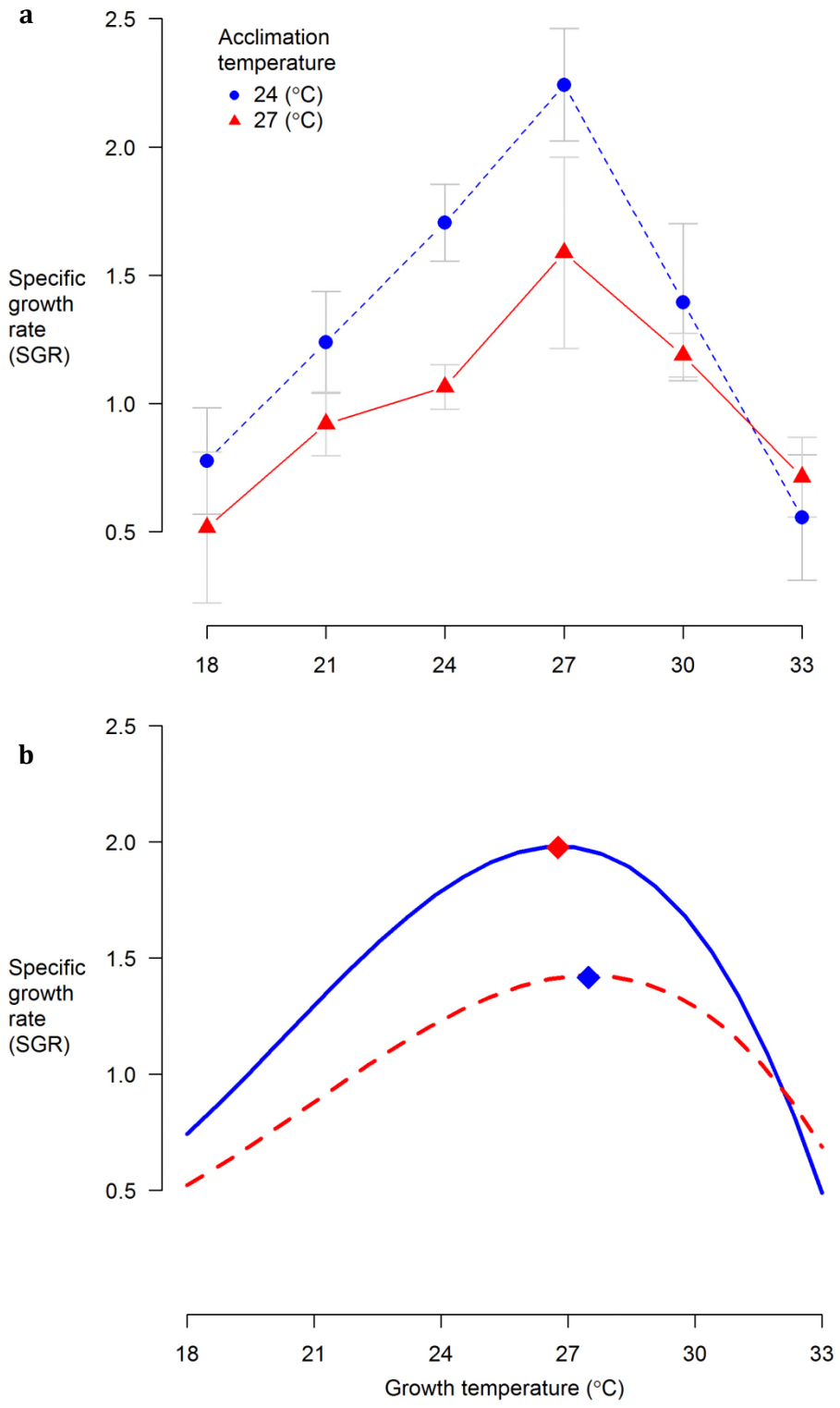


Figure 3: a) Average newborn growth (± 1 standard error) over a two week period at each test temperature for the two acclimation temperatures. **b)** Third order polynomial curve describing the relationship between growth and temperature for offspring born into each acclimation treatment. The diamond indicates the T_{opt}, for each treatment, red diamond (24^{0}C treatment) = 26.8^{0}C, blue diamond (27^{0}C treatment) = 27.5^{0}C).

Juvenile growth:

The fish added to the growth tanks were aged between 0-14 days old and, consequently, variable in their sizes. By 11 weeks, fish were larger in the 27^0C temperature tanks regardless of acclimation temperature although growth was fastest in the treatment combining lower developmental temperature and higher growth temperature **(Table 6)**. Growth was slower for fish held at 24^0C and there was no difference in growth trajectory regardless of acclimation treatment. From five weeks of age the pattern of growth – LH largest, HH second with HL and LL smallest – was consistently maintained until the end of the experiment **(Figure 4)**.

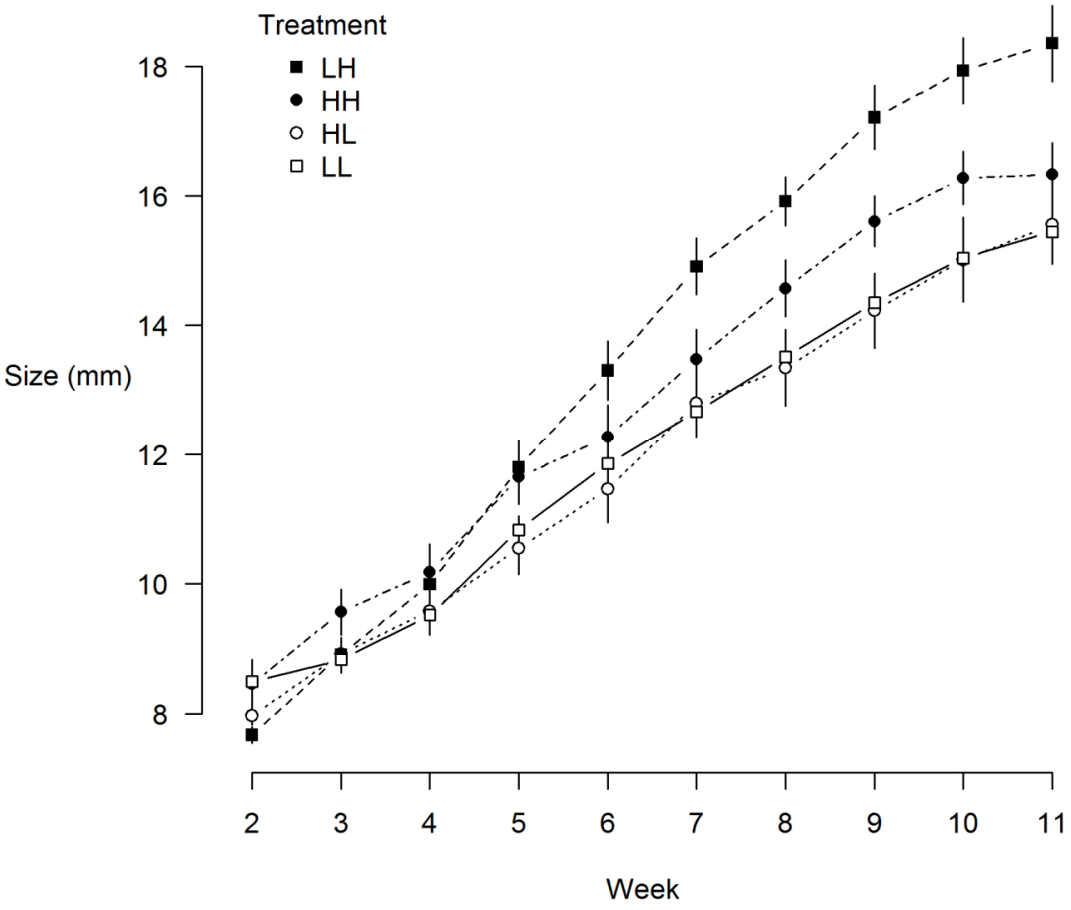


Figure 4: Change in size over time for fish from the four treatment groups, each point is the average of three replicates ± 1 standard error. Filled symbols represent the higher growth temperature.

Table 6: Change in size and specific growth rate (SGR) for each treatment. Numbers are grand means of all fish across replicates. Also, total number of fish entered into the experiment and mortality reported during the growth period.

Treatment	Week	Ave. Size	sd	SGR	No. fish	Mortality
HH	2	0.85	0.155		17	2
	11	1.63	0.187	0.493		
HL	2	0.8	0.061		18	2
	11	1.59	0.214	0.468		
LH	2	0.77	0.059		21	7
	11	1.84	0.22	0.61		
LL	2	0.85	0.063		21	0
	11	1.51	0.171	0.417		

The bootstrapped Von Bertalanffy parameter estimates indicated the HH treatment had the highest value growth coefficient (*k*) and fish reached an asymptote at the smallest size which also suggests reproductive maturity was reached quicker/at a younger age **(Table 7, Figure 5)**. The exact opposite was predicted for the LL treatment with the lowest value for *k* and largest asymptotic size although the model failed to predict realistic *Linf* values suggesting the majority of fish in this treatment were still growing when the experiment was terminated. Fish which developed in the lower acclimation temperature were predicted to attain a larger max size than fish from the higher regardless of growth temperature. The growth coefficient was similar for both of the crossed treatments suggesting an effect of changes to thermal regime during and after development. Because of the sex differences in guppy growth the confidence intervals surrounding the parameter estimations are very large and it is impossible to develop this tantalising result further.

Table 7: Parameter estimates and 95% confidence intervals for fish exposed to each treatment. *Linf* is the theoretical maximum size and *k* is speed at which fish approach that size.

	Treatment			
	LL	LH	HL	HH
Linf	7.563	2.885	2.391	2.266
2.50%	3.232	2.379	1.895	1.929
97.50%	14.592	4.238	4.743	3.486
k	0.014	0.078	0.074	0.094
2.50%	0.006	0.041	0.024	0.041
97.50%	0.044	0.119	0.131	0.144

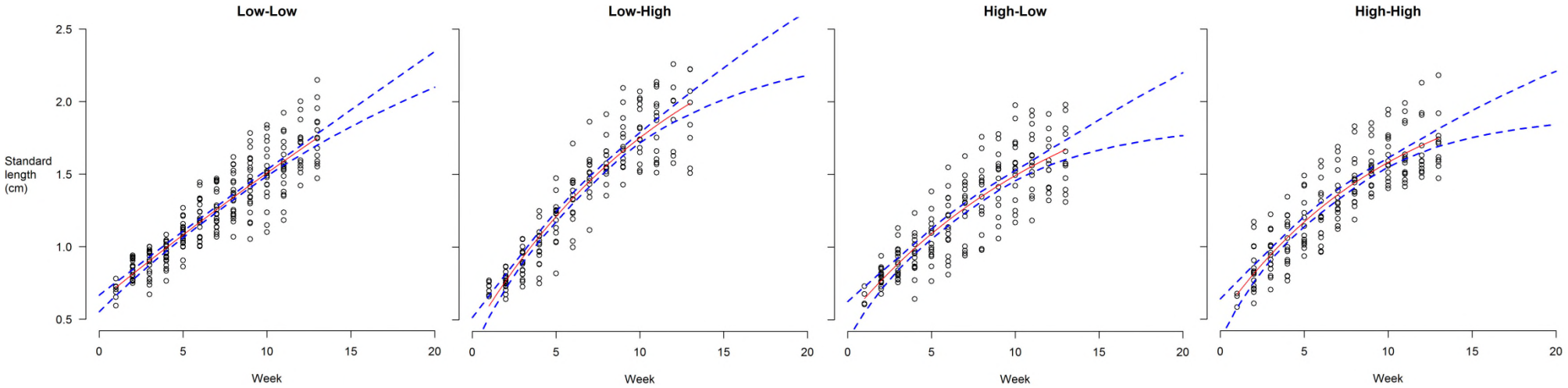


Figure 5: Growth curves of the fish from each treatment. Fish were measured every seven days and so the points are repeated measures of the same fish. The red line is the fitted Von Bertalanffy model; the blue dashed line is the predicted confidence intervals for length at age based on the bootstrapped results.

Discussion

The results from these experiments demonstrate that guppies are able to partially acclimate to novel temperatures and that a combination of developmental and environmental temperature affect important life history parameters with potential long term fitness implications.

Acclimation:

Acclimation was seemingly not achievable at the highest temperature. Despite guppies routinely experiencing temperatures greater than 30^0C in the wild **(Chapter 2)** and the aquarium fish continuing to perform mating behaviours when exposed to >30^0C **(Chapter 3)** the upper temperature in this experiment was too stressful for prolonged exposure. As a general response to temperature fish held at higher temperatures had higher mortality and smaller offspring with fish at the highest temperature stopping production of offspring entirely. This indicates that guppies are able to withstand periodic exposure to elevated temperatures however continuous exposure is detrimental. This conclusion supports a recent study which demonstrated that elevated temperatures have negative physiological effects on guppies with a critical threshold affecting physiological condition at temperatures around 30^0C (Muñoz et al. 2012).

Increases in temperature are closely related to an increase in metabolism and a resultant increase in toxic reactive oxygen species (ROSs) by-products (Hemmer-Brepson et al. 2014) and there is evidence that acclimation may allow for adaptive mitigation of oxidative stress through upregulation of ROS scavenging enzymes (Selman et al. 2000). It may be that the fish in the 30^0C treatment lacked

the mitigating methods to survive in such temperatures for extended periods. Similarly in environments where temperature fluctuates over a regular daily cycle organisms may be able to adjust behaviourally and perform metabolically costly activities at lower temperatures to minimise the damage caused by high temperatures (Greenwood and Metcalfe 1998, Johnston et al. 2004, Kearney et al. 2009). Alternatively reproductive rates and lifetime fecundity can actually be higher in fluctuating conditions allowing organisms to compensate for the negative effects of elevated temperatures (Folguera et al. 2011). Consequently, in **Chapter 5 and 6** I investigate guppy growth, reproduction and secondary sexual traits in fluctuating thermal regimes.

Mating behaviour:

Mating behaviours broadly followed the beneficial acclimation hypothesis which states that organisms will perform better when tested at the temperature closest to their recent thermal experience (Wilson and Franklin 2002). Mating behaviour was variable between individuals, however, and recorded across the entire range of test temperatures (23-33^{0}C) for both 24^{0}C and 27^{0}C treatments. Generalised linear models indicated that the interaction between acclimation and test temperature was integral to describing the mating behaviour although only display behaviour was well explained by modelling and included male and female size as important covariates. Sneak mating, following time, distance travelled and coefficient of variation (CV) in swimming speed were not well described by my modelling approach with very little of the residual deviance explained **(Table 8).**

Further replicates may have clarified the relationship, although as mating behaviours have already been demonstrated to be variable and resilient to thermal change **(see Chapter 2 and 3)** this absence of pattern may have been observed regardless of any increase in replicates. The temperatures I employed may have all been within a relatively optimal part of the guppy's mating thermal performance curve (TPC). Extending the temperature range may have been more challenging to the guppy and enabled me to capture the upper and lower limits of the TPC. Conversely, other reproductive traits such as male colouration and sperm morphology may be more sensitive to warming temperatures (e.g. Breckels and Neff 2013) and be better targets of investigation **(see Chapter 5 and 6)**.

Juvenile characteristics:

Offspring number and size at birth:

At the intermediate temperature, total fecundity was not dissimilar to the lower (24^0C) treatment although in hotter treatments offspring became progressively smaller over time before fish in the 30^0C treatments stopped producing offspring entirely. The relationship between temperature and offspring size is consistent with the *temperature-size* rule (Atkinson 1995) although theory predicts that smaller offspring will be produced in higher numbers (Smith and Fretwell 1974); a result that I did not observe. It could be that 27^0C is not so metabolically costly as to restrict maternal investment to the point where offspring number decreases. It would be interesting to see for how long the 27^0C offspring continued to decrease in size until the point where offspring size either plateaued; production stopped entirely; or fecundity became significantly different.

Theory predicts that the optimal approach for mothers is to adjust their reproductive investment according to the environmental conditions their offspring are likely to encounter and that larger offspring have advantages in adverse conditions (Marshall and Uller 2007). For wild guppies, the main predators are gape limited to quite small fish and so larger offspring reach a safe size much more quickly however in an environmentally benign predator-free lab environment larger offspring size may not be as important (Allen et al. 2008).

Newborn growth:

In the first two weeks from birth there was a similar pattern in specific growth rate at each test temperature regardless of acclimation temperature. There was, however, an interaction between developmental temperature and environmental temperature affecting the elevation of newborn growth resulting in fish from the colder treatment exhibiting a faster growth rate at each test temperature except at the upper extreme of 33^0C. The T_{opt} for growth rate was $\sim 27^0$C for both treatments which indicates an effect of both developmental and environmental temperature and potentially the thermal experience of the offspring's mother although it is also possible that there is a genetic component to the guppy's growth pattern (Reynolds and Gross 1992, Dmitriew 2011).

Juvenile growth:

Both thermal history and environmental temperature were important in defining juvenile growth parameters and the effect was apparent despite the variation in size at birth. Following theoretical predictions, growth was faster at higher temperatures but final size was bigger in fish from lower developmental

temperatures. Interestingly, based on the first 11 weeks of growth, fish from the LL and HL treatment had similar growth trajectories despite very different predictions for maximum size. This suggests that some combinations of thermal history and environmental temperature have stronger effects than others and that predicting whole life growth using models provide a more accurate picture than solely plotting the data. There was a great deal of variability in the size data behind the growth curves which was due to sex differences in growth as well as the variation in sizes of fish as they entered the experiment; precise size at age measurements and individually housed fish would provide clearer trends and reduce the size of the confidence intervals around the Von Bertalanffy parameters. It is also likely that the experiment was terminated too soon for the LL treatment as the growth curve was still in the accelerating phase and resulted in the wildly unbelievable confidence intervals for *Linf* in this treatment.

The temperature difference between 24^0C and 27^0C is relatively small and the shape of the guppy's thermal performance curve may be such that they exhibit similar performance levels at both of these temperatures. An early study of growth in guppies showed that growth was similar in fish born and raised at 23^0C and 25^0C degrees and both were faster than 30^0C (Gibson and Hirst 1955). Because of the asymmetric shape of thermal performance curves and the consequent rapid loss of fitness at temperatures just a few degrees higher than the optimum temperature, organisms are predicted to maximise lifetime fitness by situating their thermal preference a few degrees below their optimal temperatures (Huey and Stevenson 1979, Martin and Huey 2008). It is therefore possible that for our fish, 27^0C actually represents optimal growing conditions for guppies that have

been maintained at around 24^0C for multiple generations which supports the *warmer is better* hypothesis.

Conclusions:

In this collection of experiments I observed changes in life history variables caused by both environmental and developmental temperature and that thermal acclimation has the potential to mediate the responses. While a stable 30^0C temperature regime led to high mortality it may be that 24^0C and 27^0C degrees results in similar performance outputs for the guppy and a broader range of temperatures would have captured information on what happens at the TPC limits. It remains unclear though as to whether there are any costs that can explain the observed differences in offspring size or growth, for example in sperm characteristics or fecundity and so longer duration experiments may be more informative. Furthermore, water temperatures in natural systems are not stable and exhibit variability over daily, seasonal or annual time scales (Caissie 2006). In order to reach accurate, ecologically relevant conclusions realistic thermal regimes should be used when investigating the ecological effect of temperature (Niehaus et al. 2012). Consequently in **Chapters 5 and 6**, I explore the effects of thermal regime on growth and reproductive traits using fluctuating temperatures.

Table 8: Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1. ns = non-significant but present in the final model, NA = not included in 'best' model. CV = Coefficient of Variation in swimming speed, Mdist and Fdist = Distance travelled by the male and female over the duration of the observation.

	Response variable						
	Male CV	Female CV	Mdist	Fdist	Display	Sneak	Follow
(Intercept)	**	ns	*	*	ns	*	*
Acc temp (27)	***	ns	.	.	**	**	*
TestTemp	NA	NA	NA	NA	NA	NA	*
$TestTemp^2$	***	ns	*	*	ns	*	NA
$TestTemp^3$	NA	NA	NA	NA	NA	NA	NA
HomeTemp27 * TestTemp	NA	NA	NA	NA	NA	NA	*
AccTemp27 * $TestTemp^2$	***	ns	.	.	*	**	NA
AccTemp27 * $TestTemp^3$	NA	NA	NA	NA	NA	NA	NA
MaleSize	NA	NA	NA	NA	***	*	NA
FemaleSize	**	NA	ns	NA	**	ns	NA
MaleSize * FemaleSize	NA	NA	NA	NA	NA	ns	NA

Chapter five: The effect of thermal regime on growth and offspring phenotype

: ecologically-realistic thermal regimes have immediate effects on offspring size & growth which disappear in the next generation… #Responsive

Lay summary

Experiments investigating the effect of temperature on organisms commonly use stable temperatures in order to remove sources of variability and therefore be more confident in their results. The outcomes of many of these experiments are to be used to predict the potential effects of changing environmental conditions. If the experimental conditions are not ecologically realistic, however, the conclusions may not be realistic either. In order to investigate the effect of ecologically realistic fluctuating temperatures on guppies I created two thermal regimes which were similar to daily water temperature cycles in Trinidad. I then added pregnant females to each regime and recorded the growth trajectory of each of their offspring from birth to maturity. I then repeated the experiment with the offspring born to the females which matured in the first generation. By individually-housing each fish I was able to follow the effects of thermal regime on pregnancy and growth characteristics of each fish and by splitting family units between the thermal regimes I could investigate the effect of temperature at different ontogenetic stages – embryo development and juvenile growth. Fluctuating temperature had an immediate effect resulting in offspring born in the warmer treatment to be smaller. There was little difference in male growth between treatments but female fish in the warmer treatment are significantly smaller than their cooler counterparts. In the following generation, however, there were only minimal differences in offspring size and growth suggesting that the guppies had invested energy in growth at the expense of some other – unrecorded – life history trait.

Abstract

Organisms naturally experience fluctuation in temperatures whether over daily, seasonal or annual time scales. Therefore in order to make more ecologically realistic predictions about the impacts of climate change, species invasion or environmental disturbance studies should incorporate temperature variation in their experimental design. I exposed guppies to one of two thermal regimes in which temperature followed a repeating daily cycle similar to patterns experienced by guppies in Trinidad. Individually housing experimental fish enabled me to gather weekly measures of individual growth, which were then modelled using the Von Bertalanffy growth equation. Alongside growth I also recorded the effects on reproductive traits and offspring characteristics over successive generations. Size at birth and maximum size was smaller in warmer temperatures although fecundity and gestation time did not differ between the two regimes. By the second generation there was an increase in fecundity associated with the warmer treatment and offspring born into the cooler treatment were marginally larger. Gestation time, maximum adult size and growth coefficient were similar across treatments resulting in similar growth trajectories for fish regardless of thermal experience. This experiment is one of only a few studies to have investigated the effect of variable temperatures and how they affect life history characteristics over generational timescales. The lack of discernible difference in offspring size and growth in the second generation suggests an energetic trade-off is being made to maintain growth at the expense of some other – as yet unidentified – trait(s). Regardless of any trade-off the guppies in this experiment demonstrated that, when faced with novel conditions, they can reproduce successfully and produce offspring which are capable of reaching sexual maturity thus ensuring immediate survival.

Keywords

Variable temperature – growth – Von Bertalanffy equation – developmental plasticity – offspring size – fecundity

Introduction

Plants and animals that are subjected to fluctuating temperatures in their natural environment are more commonly thermal generalists (Kassen 2002) and this variability may be an important component in evolving tolerance to novel environmental conditions (Ketola et al. 2013). Disturbed or fluctuating environments are also cited as a common factor increasing the invasibility of species by either selecting for organismal flexibility or the evolution of 'evolvability' (Lee and Gelembiuk 2008). Furthermore, evolution in variable environments can result in broad thermal tolerance in a number of ways; by increased expression of heat shock proteins (Ketola et al. 2004); through the selective pressure of thermal variability causing energetic efficiency or better growth and viability (e.g. Parsons 2005); or the maintenance and accumulation of genetic mutations (Lee and Gelembiuk 2008).

Whilst leading to broader thermal tolerance, fluctuating temperatures can also result in poorer fitness as a consequence of decreased energetic conversion efficiency (Spigarelli et al. 1982), higher oxygen consumption (Lyytikainen and Jobling 1998), reduced heat shock protein expression (Fischer et al. 2011b) and increased standard metabolic rate (Beauregard et al. 2013) each of which can impact on resource allocation or growth.

Sub-optimal thermal conditions reduce the amount of total energy available resulting in a trade-off between growth and reproduction (Kozlowski 1992, Folkvord et al. 2014). The *temperature-size* rule (Atkinson 1994, Atkinson and Sibly 1997), which encapsulates this trade-off, states that ectotherms inhabiting

colder environments generally mature later, at a larger size and give birth to fewer, but larger, offspring than those in hotter conditions. There are, however, exceptions to this rule and evidence to suggest a positive relationship between increased temperature and growth efficiency (Angilletta and Dunham 2003). The effect of fluctuating temperatures on growth has likewise had a mixture of responses and there is evidence that fluctuating temperatures can lead to improved growth (Sadati et al. 2011) no effect on growth (Arias et al. 2011) or smaller sizes at maturity (Dhillon and Fox 2007) than purely stable temperatures.

In the wild, temperature naturally fluctuates daily, seasonally or annually and there is evidence to demonstrate that the responses of organisms to variable temperature regimes differs from their responses to stable temperatures (e.g. Kingsolver et al. 2009, Paaijmans et al. 2013). The effect of fluctuating temperatures is dependent upon the broadness of the temperature range with wider fluctuations leading to higher phenotypic flexibility (e.g. Měráková and Gvoždík 2009). Similarly, stochastic temperature changes with high variability resulted in a higher critical thermal maxima (T_{crit}) but smaller body size than variable and stable temperatures with the same mean (Schaefer and Ryan 2006). This suggests that it may be the temperature extremes or a broad thermal range that acts as the selective force on thermal reaction norms rather than the variation itself (Kingsolver et al. 2009, Bozinovic et al. 2011). The detrimental effects of elevated temperatures as a result of increased oxidative stress has also been linked with shorter lifespan and therefore total lifetime fecundity (Hemmer-Brepson et al. 2014) and there may well be other costs of thermal adaptation at a cellular level which are currently unidentified but have a direct impact on function and fitness

(Clarke 2003). The direction and strength of pressure on selection depends on more than just the range of temperatures, however. A multivariate landscape which includes the duration and scope of within- and between- generation variability; the regularity of fluctuations; as well as knowledge about the environment are also cited as important factors (Gilchrist 1995, Gabriel et al. 2005, Lee and Gelembiuk 2008).

In **Chapter 4** I demonstrated that the effect of prolonged exposure to stable high temperatures – that are routinely encountered in the wild – can have negative effects on guppies. I have also shown that temperatures in the guppy's native habitat vary considerably over a repeated daily cycle and this variability represents a source of selective pressure potentially responsible for the guppy's broad thermal tolerance **(Figure 1, Chapter 1, Appendix 1)**. I therefore wanted to investigate the effect of ecologically realistic, variable temperatures, including those which are damaging under prolonged time scales, on the growth and offspring of guppies. Accordingly, in this chapter I ask the question:

What are the effects of environmentally-realistic fluctuating temperatures on growth and reproductive characteristics?

Prior to maturity, energy allocation is prioritised for somatic growth and maintenance while post maturity a much larger proportion of resources are divested towards reproduction as growth slows. The majority of fish (as well as numerous invertebrate and vertebrate groups) follow this pattern of indeterminate growth whereby they continue to grow after maturation, albeit at a

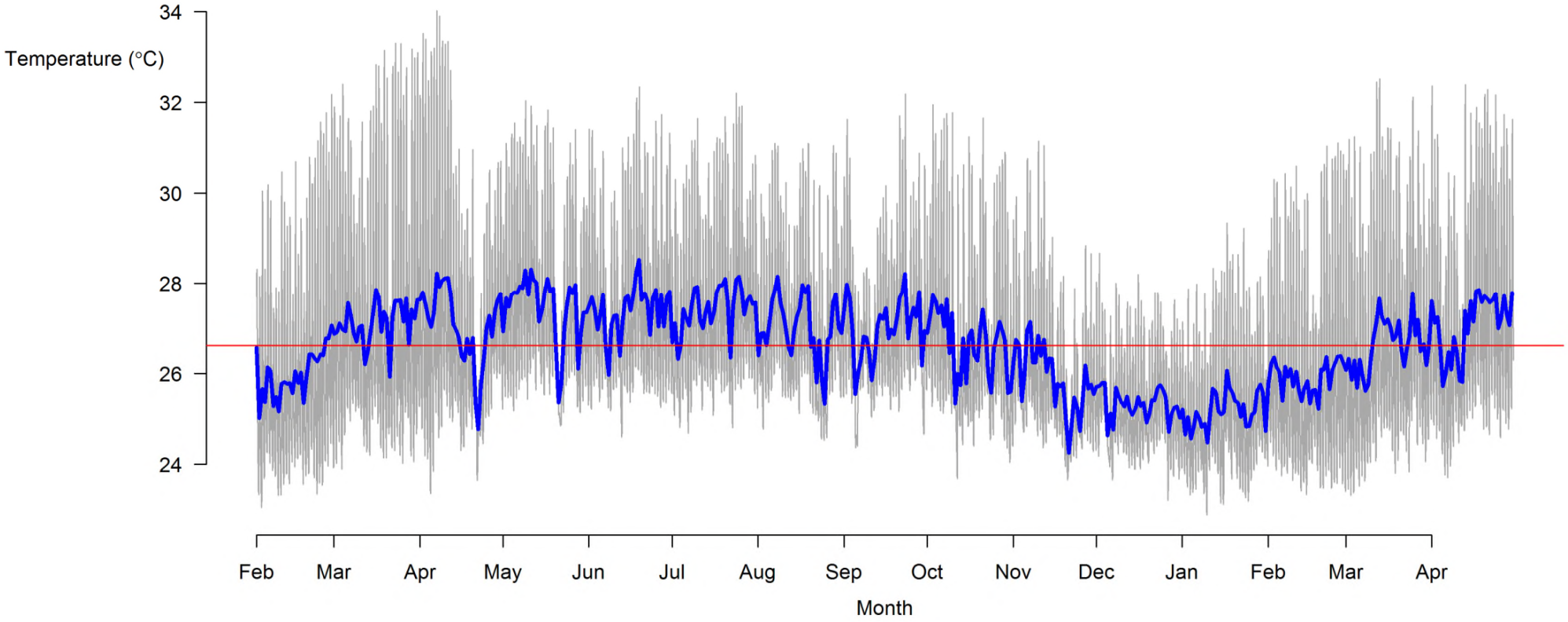


Figure 1: Daily variation in water temperature (grey line) in the Acono River in Trinidad at a site where guppies are found. Blue line is the daily mean and red line is the overall mean. Temperatures were taken every 30-60 minutes using a data logger (MicroLite lite5016, Fourier Systems).

slower rate than before maturing (Heino and Kaitala 1999). As fecundity and offspring size both increase with increasing female size (as well as the associated competitive and predator avoidance benefits) it is advantageous for female guppies in favourable environments to be larger. However environmental conditions (Breckels and Neff 2013), resource availability (Bashey 2006) and social environment (Bashey 2008) experienced by the mother all influence the size and number of offspring guppies produce for each brood resulting in females adjusting their reproductive investment based on current conditions. Male guppies, conversely, tend to divest very little energy to growth post maturation and allocate most of their energy to reproduction and foraging although the larger the size at which guppies reach maturity the better the fitness benefits (Reznick 1990).

Temperature has powerful effects on organismal biology at all stages of ontogeny although commonly it is the temperatures encountered during development which have permanent effects on an organism's phenotype (Wilson and Franklin 2002). Developmental rate is controlled by temperature and the timing of environmental fluctuations during development, the magnitude of fluctuation, and whether it is close to the lower or upper physiological tolerance limits of an organism alters morphological and physiological traits differently (Spicer and Burggren 2003, Arrighi et al. 2013). These changes in traits can result in fitness consequences and provide the variation on which evolution can act (Moczek et al. 2011, Spicer et al. 2011). Phenotypes that are fixed as a result of gene expression during development can have long term fitness consequences affecting gender determination (Ospina-Álvarez and Piferrer 2008), the duration of

life stages and predator vulnerability (Touchon and Warkentin 2010) or growth and swimming ability (Scott and Johnston 2012). It is therefore advantageous in fluctuating conditions for plasticity to be reversible and allow for broad environmental tolerance (Gabriel et al. 2005).

This series of experiments investigates the phenotypic changes fish make when exposed to completely novel, environmentally-realistic conditions during different stages of ontogeny: embryo development, juvenile growth and maturity.

In **part 1**, I asked what are the effects of changing thermal regime on guppy pregnancy characteristics and offspring size?

My aim was to determine the immediate consequences of entering a novel thermal regime (for example when moving up or downstream, or being released in a new area). Female fish with a shared thermal history – and consequently similar reproductive investment – were mated and placed into a novel thermal regime. Gestation time, fecundity, growth while pregnant and offspring size were then recorded. Following theoretical predictions I expected the warmer regime to result in shorter gestation time, higher fecundity and smaller offspring size at birth.

In **part 2**, I asked what are the effects of developmental and growth temperature on the growth and adult size of guppies?

The aim of this part of the experiment was to separate the effects of *developmental* and *growth* temperature on growth trajectories. The offspring born to the fish described above were split between thermal regimes and individually housed which allowed me to take weekly size measurements of each fish. I predicted that fish from the cooler developmental conditions would attain a larger

size and that fish in the warmer conditions would grow more quickly and mature at a smaller size than those in the cooler environment.

In **part 3** I asked what are the effects of developmental and growth temperature on guppy pregnancy characteristics and offspring size?

The aim of this experiment was to investigate the effect of maternal thermal experience and offspring developmental temperature on gestation time, fecundity, growth while pregnant and offspring size. I predicted that fish which have been held in the hotter thermal regime across generations would have a shorter gestation time and smaller offspring than the reciprocal fish from the cooler thermal regime. I further expected fish with a mixed thermal history to be more variable and intermediate between the two consistent exposure regimes as in unpredictable environments variability in response is favoured (Fischer et al. 2011a).

In **part** 4 I asked what are the effects of developmental and growth temperature, as well as the mother's thermal experience, on the growth and adult size of guppies?

The offspring resulting from the previous experiment were split between thermal regime and their growth was recorded weekly until post maturity. Individually housing the fish allowed me to investigate possible transgenerational effects of the mother's developmental temperature as well as the offspring's developmental and growth temperature. Again I predicted fish in the hotter conditions would grow more quickly and mature at a smaller size than those in the colder environment. Furthermore I expected fish from the consistently higher and lower temperatures to attain the smallest and largest sizes respectively.

Methods

Temperature regimes and systems maintenance

Six (eight in generation two) 135 litre plastic water baths were filled with aged tapwater and fitted with an aerated bio-mechanical filter and pump (Maxi Jet Ltd.) to circulate the water. Each bath was assigned to one of two thermal regimes ("colder" and "hotter") which were created using 300 watt aquarium heaters (Deltatherm, Interpet Ltd.) controlled by timer switches. The daily temperature cycles were similar to those found in Trinidad at a pair of sites under 10km apart on the Acono River where guppies are present **(Figure 2a)**. The "hotter" regime included routine exposure to temperatures apparently stressful to guppies **(see Table 1 and Chapter 3)**. Water temperature was recorded every 30 minutes in each water bath using temperature data loggers (MicroLite lite5016, Fourier Systems) and followed a regular daily thermal profile **(Figure 2b & c)**. Aged tapwater was added to each bath weekly to maintain a uniform depth and the water chemistry of each water bath was tested periodically. The aquarium was subject to a 12:12 light:dark cycle and throughout the experiment all fish were fed to excess daily with either commercially available flake food or *artemia* nauplii.

Fish size was measured using the *ImageJ* image analysis software (www.imagej.nih.gov/ij/) from photographs taken of each individual alongside a scale.

Table 1: Temperature characteristics of experimental thermal treatments and reference sites at two locations within the same river in Trinidad

	Colder	Hotter	Upper Acono	Lower Maracas
Mean	25.04	27.41	24.63	26.62
Median	25.17	27.20	24.39	26.18
sd	1.56	1.68	0.80	1.96
Range	10.92	10.87	4.41	11.14
Min	19.14	22.05	22.99	22.88
Max	30.06	32.92	27.4	34.02
Count	46963	47516	1722	10886

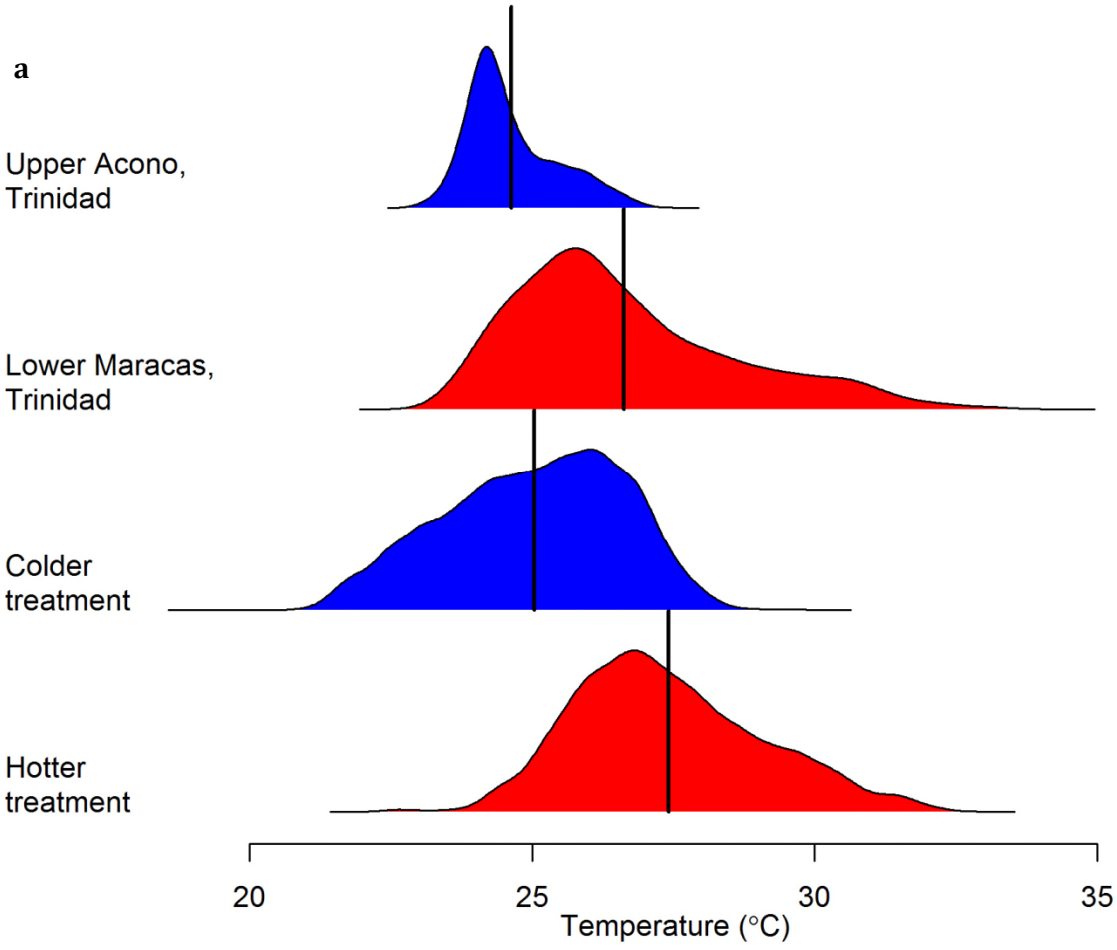
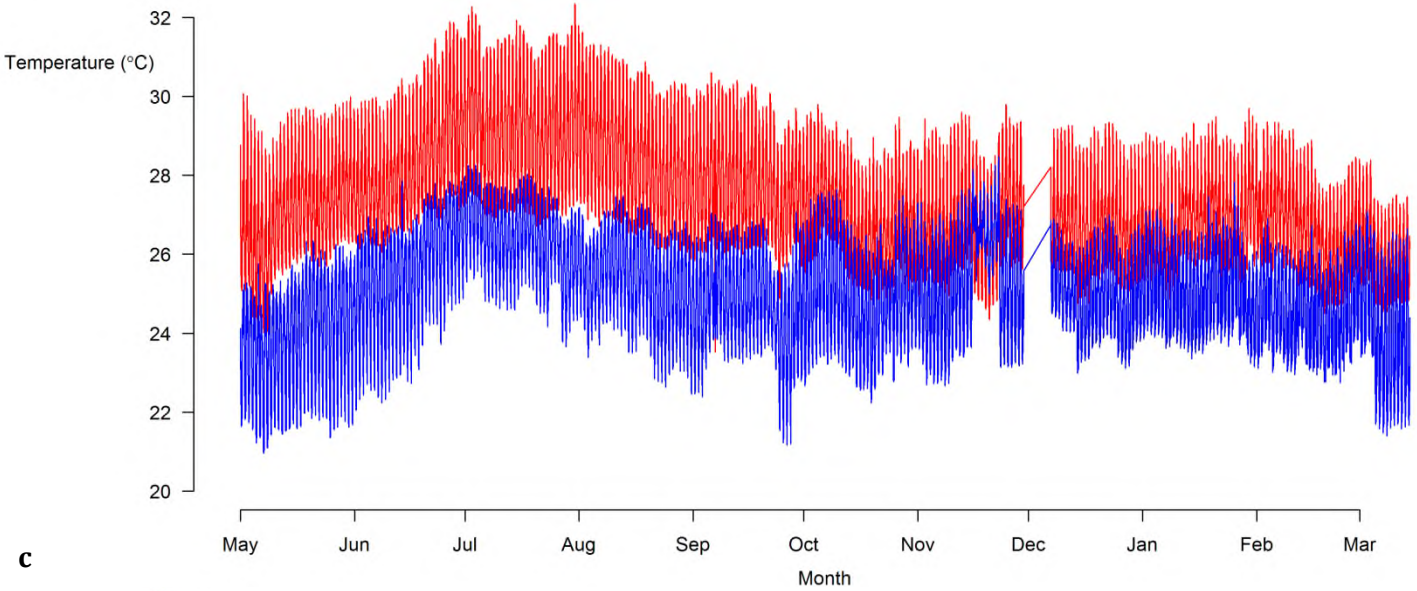


Figure 2: a) Density plots of experimental temperature regimes recorded over the duration of the experiment and in reference locations in Trinidad. Upper Acono and Lower Maracas are sites on the same river separated by ~10km. Black vertical bar is the mean value. **b)** Mean temperature across thermal treatment in hotter (red) and colder (blue) tanks over the duration of the experiment. **c)** Example of the daily cycle of temperature experienced in individual water baths in the hotter (red) and colder (blue) temperature treatments

b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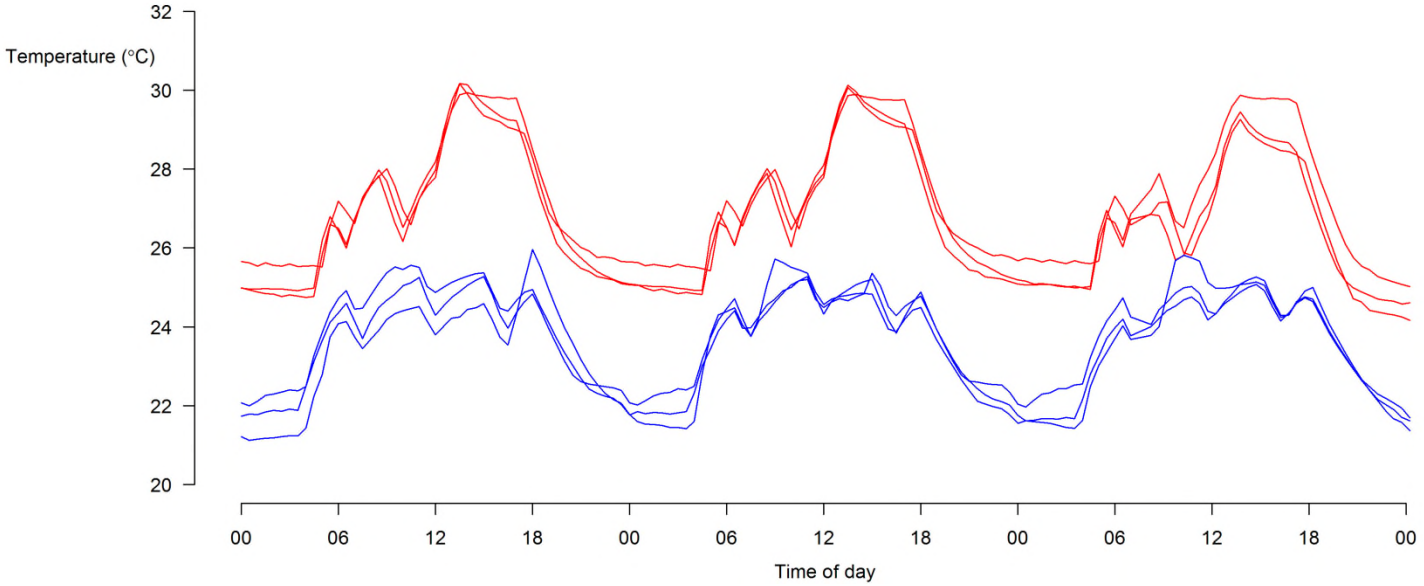
Temperature (°C)
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Time of day

Figure 3: Experimental system. Water baths with temperature controlled by aquarium heaters plugged into timer switches. Fish were housed individually in transparent hatchery tanks to allow visual interaction.

Experimental fish – founder population

Descendants of fish originally from the Lower Tacarigua River (Trinidad) and housed in our aquarium for multiple generations were grown until maturity at ambient temperature (~24^0C ±1.35 sd). When fish exhibiting male characteristics were first spotted (the first appearance of colour and the elongation of the pelvic fins forming the gonopodium) they were removed leaving the females to mature as virgins.

These mature females were photographed with a scale (for size measurements) and placed separately into 15 litre mating tanks (females n=65, mean = 1.62, range = 1.21-2.23, sd = 0.21). Mature male fish from stock tanks were photographed and one was added to each of the mating tanks (males n=70, mean = 1.54, range = 1.32-1.8, sd = 0.122). Each female was exposed to one male for 24 hours before males were circulated along one tank and left to interact with a female for a further 24 hours. This was done a third time allowing a female 72 hours to mate successfully. After three days females were assigned a temperature treatment ("*colder*" or "*hotter*") and transferred to a floating fish hatchery (Marina, Hagen Inc.) within one of the experimental water baths until offspring were born. Hatcheries are transparent (allowing social interaction between fish) and have narrow slits on two sides to allow water flow in and out allowing waste to be cleared and chemical cues to circulate **(Figure 3)**.

Hatcheries were checked daily for juveniles. When offspring were observed they were photographed, assigned a thermal regime and housed in individual hatcheries. One brood and a maximum of six offspring per female were used (three

per thermal regime) until there were ~60 juvenile fish per *hotter* and *colder* treatment **(Table 2)**.

After either giving birth; two months without giving birth; or when enough juveniles per treatment had been produced females were photographed and removed from the experiment. From these fish I could measure *gestation time*, *fecundity* and the *specific growth rate* (SGR) of females from size at fertilisation until size at time of giving birth. A total of 64 females were mated and placed in the thermal regime 35 of which provided 130 offspring for the experiment **(Table 4)**.

Experimental fish – generation 1

Each offspring was photographed for size measurement every proceeding Friday and replaced in a different location within a different water bath to remove any possible tank effects. This was repeated for between 13-19 weeks.

Table 2: The numbers of fish entered into each treatment in generation 1. Offspring born to a female were split between thermal regimes (when more than 1 was born) and number of families represents the number of females that provided offspring for each treatment.

Embryo temperature	Growth temperature	Treatment code	Number of fish	Number of families
Hotter	Hotter	HH	31	15
Hotter	Colder	HC	31	17
Colder	Hotter	CH	34	17
Colder	Colder	CC	34	17

The fish within this F1 generation all originated from eggs which were produced at a common temperature. Embryos developed once females were mated and placed into one of the two thermal regimes (hereafter "*embryo temperature*")

and the offspring – born in one thermal regime – were split equally between the two regimes (hereafter "*growth temperature*") which resulted in four thermal treatments. This part of the experiment compares the effect of developmental and growth temperature.

To found generation 2, females were allowed to mate with a male from the same treatment for 24 hours before being presented with a different male for 24 hours and a third for a further 24 hours (each from the same treatment). Pregnant females were replaced individually into floating hatchery tanks which were checked daily for the presence of offspring. Once observed, offspring were removed and they and the female were photographed individually.

As with the founder fish, *gestation time*, *fecundity* and *SGR* of the each female was recorded. In total 67 females were mated, 52 of which provided the 208 offspring used in this half of the experiment **(Table 4)**. This part of the experiment compares the effect of developmental and growth temperature as well as the thermal experience of the mother.

Experimental fish – generation 2

Two further 135l water baths were set up, one for each temperature regime, to create more space to house fish. After photographing, offspring were split between the two thermal regimes which resulted in eight thermal treatments considering their *embryonic*, *growth* and *mother's embryonic* temperature **(Table 3)**. Size measures were taken on a weekly basis following the same method as generation one for up to 20 weeks.

During both generation 1 and 2 fish which either escaped their hatcheries or died were recorded and removed from the experiment. This accounted for around 10% of fish in each generation and was split fairly evenly between thermal treatments (Gen 1 colder: n = 4, hotter: n = 9. Gen 2 colder: n = 17, hotter: n = 8).

See **Figure 4** for the full experimental design.

Statistical analysis

The effect of *embryonic* and *growth* temperature on each dependent variable was analysed using a 2-way ANCOVA in R. A 3-way ANCOVA was used to assess the effect of maternal thermal experience. *Standard length* was included as a covariate to account for differences in size between fish during model selection. Model selection was made by minimising AIC (Akaike Information Criterion) values (Akaike 1973, Symonds and Moussalli 2010). Due to the small sample sizes, a derivative of AIC – AICc – was used to compare the models as it has a built in small sample bias correction (Johnson and Omland 2004).

Count data was modelled using a generalised linear model (glm) with a poisson distribution. As count data is quite frequently overdispersed, the Quasi-AIC (QAIC) value, which takes overdispersion in the form of a variance inflation factor into account, was employed in the same way as AIC where necessary (Burnham and Anderson, 2001, Richards 2008).

The D^2 value (the non-parametric equivalent of R^2) which gives a measure of the percentage variance explained by each model and adjusted D^2 (analogous to the adjusted R^2) were calculated as per the equations described in **Chapter 3**.

Specific growth rate (SGR), which measures the proportional increase in size over time, was calculated by the following equation:

Equation 1:

$$\boldsymbol{G = \left(Ln(S_t) - Ln(S_i)\right)/t}$$

Where growth (G) is a function of the log final size (S_t) minus log initial size (S_i) divided by the duration (t) between the two measures.

The effect of thermal regime on growth parameters was described using a modified Von Bertalanffy growth function **(Equation 2)** (Beverton 1954, Caillet et al 2006) and estimated using the FSA package in R (Ogle 2012).

Equation 2:

$$\boldsymbol{L(t) = L_{\infty}\left(1 - e^{-k(t-t_0)}\right)}$$

Where *L(t)* is length as a function of time and *Linf* (L_∞) is the theoretical asymptotic length. *K* is the Brody growth rate coefficient which is a value describing the speed at which the maximum size will be reached. t_0 is the theoretical time at length zero although as I recorded the date of fertilisation and size at birth of each offspring this parameter is not as useful as the actual values and will not be reported here. As the standard errors of non-linear regressions can be poorly estimated the parameters and confidence intervals were approximated with a bootstrapping routine. The data was resampled 1000 times to generate values for each of the parameters as well as providing 95% confidence intervals for the predictions.

Table 3: The numbers of fish entered into each thermal regime in generation 2. Offspring born to a female were split between thermal regimes (when more than 1 was born) and number of families represents the number of females that provided offspring for each treatment.

Mother's embryo temperature	Embryo temperature/ Mother's growth temperature	Growth temperature	Treatment	Number of offspring	Number of families
Hotter	Hotter	Hotter	HHH	22	11
Hotter	Hotter	Colder	HHC	24	10
Hotter	Colder	Hotter	HCH	32	10
Hotter	Colder	Colder	HCC	31	10
Colder	Hotter	Hotter	CHH	18	10
Colder	Hotter	Colder	CHC	18	13
Colder	Colder	Hotter	CCH	29	11
Colder	Colder	Colder	CCC	34	11

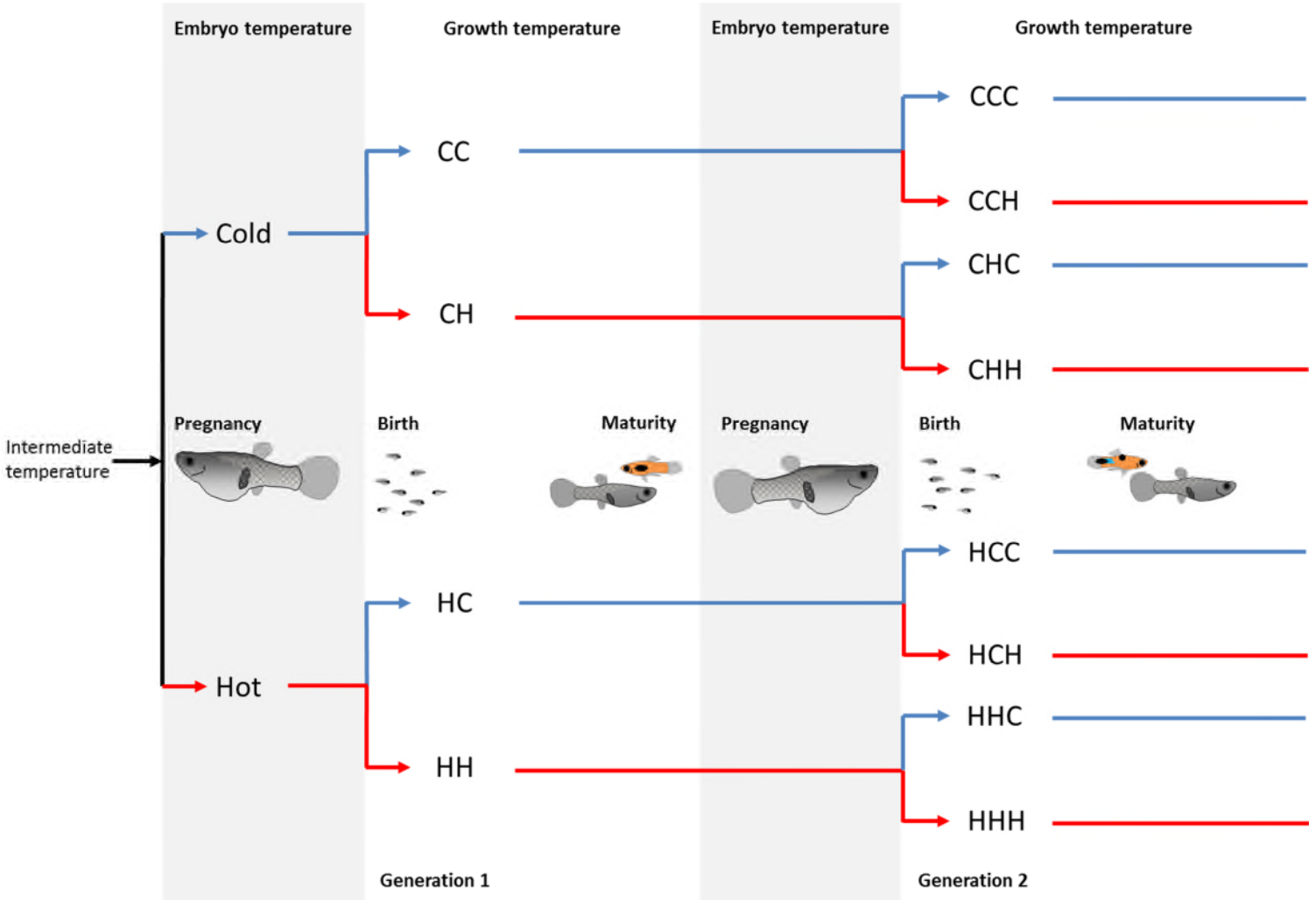

Figure 4: Experimental design. Virgin females from our lab aquarium were mated and placed into one of two thermal regimes until they gave birth. These offspring were split equally between the thermal regimes then photographed and measured weekly until maturity. One mature, females were mated with males from the same treatment and returned to their individual container until giving birth. These offspring were split between thermal regime and measured weekly until maturity.

Table 4: Characteristics, plus standard deviation (sd) and range, of fish used entered into each treatment at various stages during the experiment. For founder fish, C and H represent temperature at which fish were housed and for Generation 1 fish the letter couplet indicates *embryo* and *growth* temperature.

	Treatment					
	Founder fish		**Generation 1**			
	C	**H**	**CC**	**HC**	**CH**	**HH**
Number of females	18	17	13	13	15	11
Size when mated (cm)	1.72	1.69	1.99	1.89	1.67	1.68
sd	0.2	0.13	0.097	0.011	0.1	0.1
Gest time (days)	32.2	28.1	29.5	31.7	32.3	32.8
sd (range)	11.3 (17-68)	9.3 (17-48)	9.1 (15-43)	8.1 (22-47)	7.9 (19-50)	6.5 (22-40)
Specific growth rate (average)	0.234	0.173	0.07	0.09	0.08	0.09
sd	0.15	0.09	0.05	0.04	0.04	0.05
Number of offspring	76	59	82	88	35	46
Fecundity	4.2	3.5	6.3	6.8	2.3	4.2
sd (range)	2.5 (1-10)	2.1 (1-8)	3.4 (1-12)	3.9 (2-17)	1.6 (1-5)	1.7 (2-7)
Offspring size (cm)	0.67	0.6	0.65	0.63	0.63	0.63
sd	0.05	0.05	0.04	0.04	0.04	0.04

Results

Part 1 – Founder fish pregnancy characteristics and offspring size

The female fish used to found the two generation 1 populations (colder and hotter) were a similar size (t-test t (29.36) = 0.5069, p-value = 0.616). 70 different males were used to mate the females, average (± sd) standard length (sl) was 1.542 cm (± 0.122) range=1.324-1.8, (n = 70).

There was no significant difference in the gestation time of offspring between the two temperature regimes (t test t (32.412) = 1.1608, p-value = 0.25427). When modelled with a linear model, neither *growth temperature* nor *female size* were important coefficients and no combination of explanatory variables outperformed the null model containing no explanatory variables. The gestation time of some of the fish was much longer than can be considered one gestation cycle although separate reproductive cycles could not be clearly distinguished by histogram plots **(Figure 5)**. Gestation time can be highly variable for guppy's first brood (Evans and Magurran 2000) and the gestation times recorded here were too variable to infer discrete cycles for either temperature treatment.

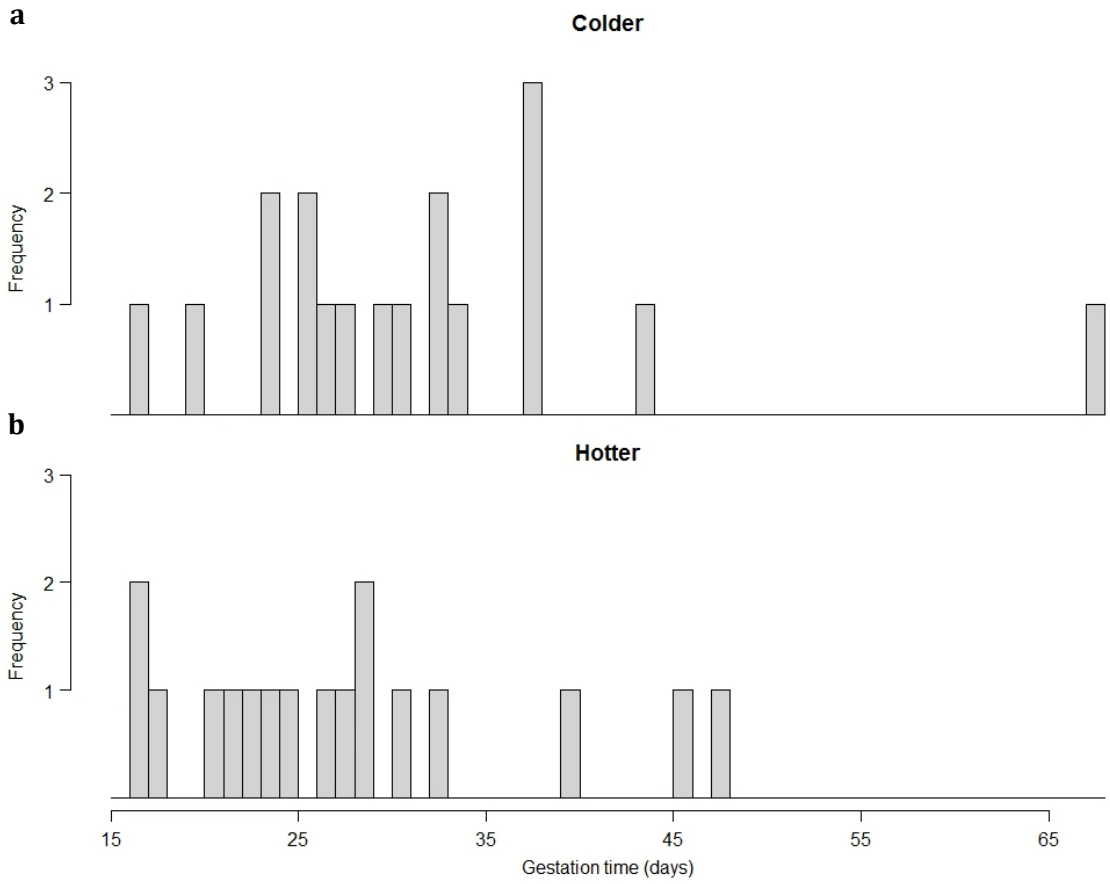

Figure 5: Histogram of gestation times for fish within either the colder (a) or hotter (b) treatments. There is no obvious pattern to indicate periodicity in reproductive cycles.

During pregnancy there was an effect of temperature, when taking initial female size into account, on Specific Growth Rate (SGR) which affected the elevation of the regression slope. Consequently, for a given size, fish in the colder treatment had a higher SGR than the hotter treatment **(Figure 6)**. For the colder treatment SGR was described by the equation 1.29 – 0.614 * size (cm) whereas for the hotter treatment SGR = 1.04 -0.51* size (cm), $R^2 = 0.915$, $F(4,31) = 83.75$, p-value < 0.001.

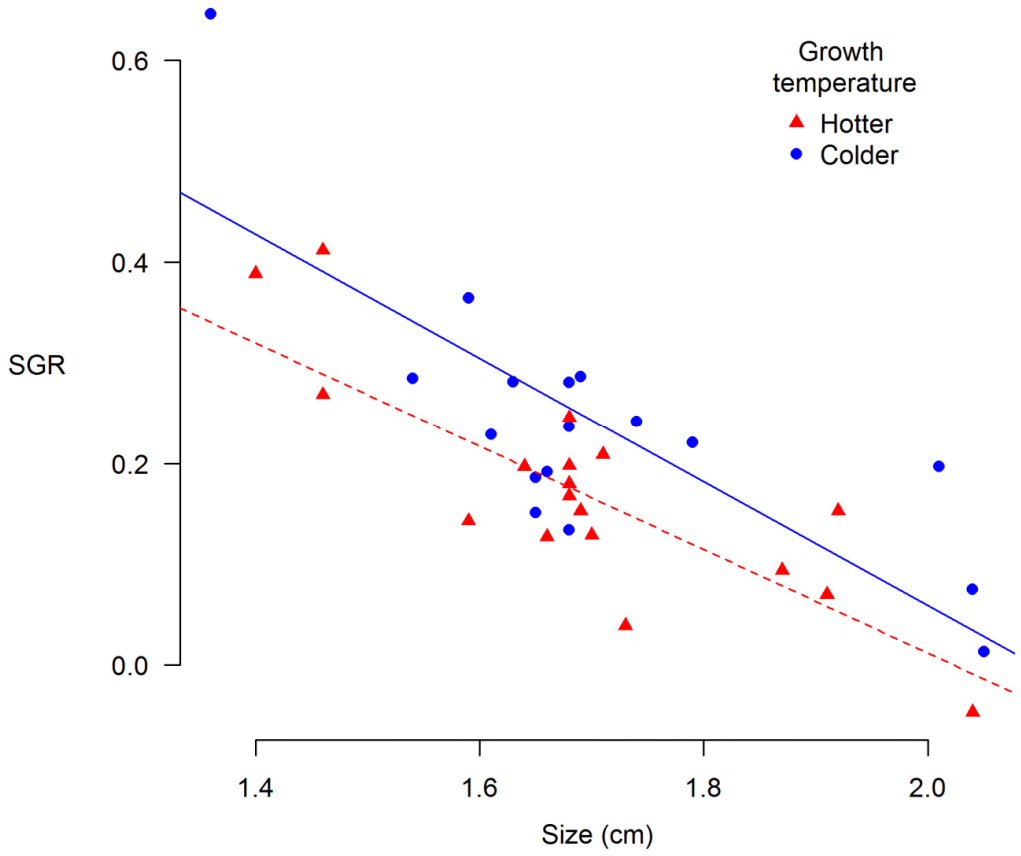

Figure 6: The relationship between initial female size and specific growth rate (SGR) in the two thermal regimes. Larger initial size resulted in lower SGR. Regardless of size, SGR was higher in colder treatments. Blue circles = colder and red triangles = hotter embryo temperature. Solid blue (colder) and dashed red (hotter) lines are linear regressions of SGR against female size.

There was no difference between the fecundity of females in either treatment (t test t (32.526) = 0.964, p-value = 0.34). Initial size of female was important in explaining most of the variation in fecundity and even when female size was included, treatment had no significant effect. Offspring size was significantly different between treatments (t test t(132.787) = -7.4304, p-value <0.001, **Figure 7**). Within the best model, offspring embryonic temperature was strongly significant (β = -0.056, t = -4.702, p-value < 0.001) although female size was not included.

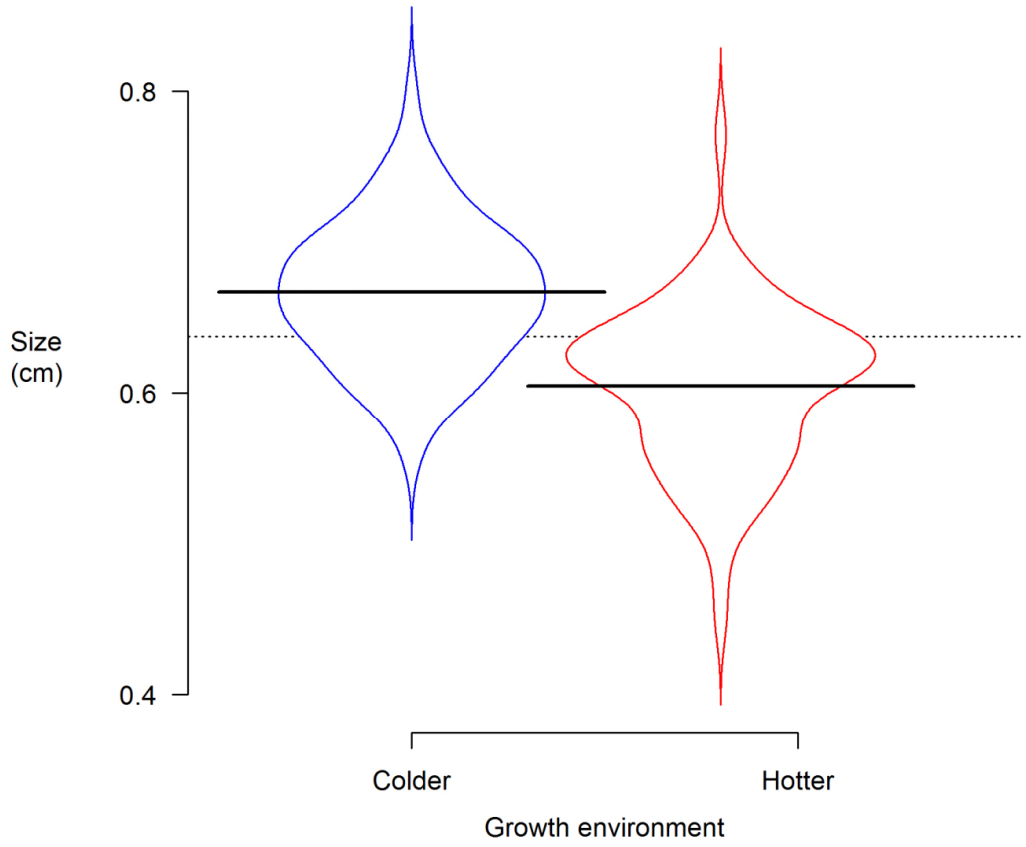

Figure 7: Beanplot of offspring sizes recorded in the two thermal treatments – colder (C) and hotter (H). Offspring size was bigger in the colder environment. Solid black line is the mean for each treatment and the dotted line is the overall mean across treatments. The size of the bean reflects the frequency of values for a given size and the shape gives a visual representation of the distribution of values around the mean.

Part 2 – Generation 1 growth and adult size

Male and female fish were similarly sized at birth (t test t (106.49) =-1.01, p-value = 0.315) and so it was impossible to predict the sex of an offspring from their birth size.

Sex of the fish, unsurprisingly, affected the *Linf* (β = -0.675, t = -17.614, p-value < 0.001) with male fish growing to a smaller maximum size than females. However there was also an interaction between sex and both *growth* (β = 0.326, t = 6.059, p-value < 0.001) and *embryo* (β = -0.094, t = -1.675, p-value < 0.1)

temperature suggesting that the effect of temperature is different for male and female fish.

Male *Linf* was smaller in when placed in hotter environments and this is supported by the best model which indicated that growth temperature (β = -0.098, t = -4.027, p-value < 0.001) was a highly significant predictor and that embryonic temperature was also marginally significant (β = -0.047, t = -1.923, p-value < 0.1) with hotter embryonic temperatures leading to smaller *Linf* (adjusted R^2 = 0.253, F (2,47) = 9.288, p-value < 0.001).

Growth temperature alone was retained in the best model describing *Linf* for females with hotter temperatures resulting in smaller maximum size (adjusted R^2 = 0.781, F (1,66) = 239.6, p-value < 0.001). The effect of growth temperature on *Linf* was much stronger in female fish as evidenced by the coefficient estimate being much larger than for males (β = -0.453, t = -15.48, p-value < 0.001). There was similar AIC support for the model including embryo temperature suggesting that embryonic temperature may be important although as the simpler model is nested within the more complex one with no significant improvement in fit (change in: R^2 = 0.0023. F = 120.9, p-value = 0) the more simple model is reported here (Richards 2008).

There were strong sex differences in *k* (β = 0.024, t = 9.982, p-value < 0.001) indicating a faster growth coefficient in male fish although this is not surprising given the sex differences in determinate (male) and indeterminate (female) growth. There was no interaction with either growth or embryonic temperature,

however, suggesting that temperature affected both male and female fish in the same “direction”.

Modelling the effects of temperature on male and female fish separately confirms this assertion as the best models for each sex contained growth and embryonic temperature and the interaction between the two.

For male fish, the interaction between growth and embryonic temperature was marginally significant ($\beta = 0.01$, $t = 1.772$, p-value < 0.1) as a result of the differences between the HC and HH treatments but neither embryonic nor growth temperature were significant in the chosen model (adjusted $R^2 = 0.078$, $F(3,46) = 2.383$, p-value < 0.1).

Contrary to the male fish, for females both growth ($\beta = 0.003$, $t = 2.342$, p-value < 0.05) and embryonic ($\beta = -0.003$, $t = -2.092$, p-value < 0.05) temperature were significant predictors as well as the interaction ($\beta = 0.003$, $t = 1.719$, p-value < 0.1) between the two. This model had a much higher adjusted R^2 demonstrating how strong the effect of temperature is on the females (adjusted $R^2 = 0.281$, $F(3,64) = 9.738$, p-value < 0.001).

The bootstrapped Von Bertalanffy values provides parameter values which take into account the individual variability within each treatment and enables 95% confidence intervals for each parameter. Female guppies naturally grow to a larger size than males and that pattern was evident here although there were differences as a result of thermal treatment **(Table 5)**.

Females grew to a much larger size in colder environmental conditions regardless of embryonic temperature although fish in the HC treatment are predicted to grow to a larger size than the CC fish **(Figure 9a)**. *K* was significantly higher in hotter conditions the HC treatment had the lowest value for *k* significantly different from the other treatments **(Figure 8a)**.

Male fish in the CC treatment grew to the largest size while the male guppies in the other three treatments had broadly similar values for *Linf* **(Figure 9b)**. *K* was not significantly different between treatments as the confidence intervals of the bootstrapped data overlapped **(Figure 8b)**.

Table 5: Growth characteristics of fish from each treatment in generation 1, values and 2.5 and 97.5% confidence intervals are the result of bootstrapping. *Linf* is the point at which fish stop growing and *k* is the Brody growth rate coefficientwhich is a measure of the speed at which fish reach *Linf*. The letter couplet indicates the *embryo* and *growth* temperature experienced in each treatment.

		Linf				***k***			
		CC	**HC**	**CH**	**HH**	**CC**	**HC**	**CH**	**HH**
		1.56	1.48	1.44	1.42	0.0431	0.0391	0.0449	0.0478
Males	2.5%	1.54	1.45	1.42	1.40	0.0398	0.0345	0.0399	0.0422
	97.5%	1.57	1.50	1.46	1.44	0.0463	0.0437	0.0498	0.0533
		2.23	2.35	1.79	1.76	0.0186	0.0148	0.0228	0.0245
Females	2.5%	2.16	2.23	1.74	1.71	0.0170	0.0127	0.0207	0.0216
	97.5%	2.29	2.48	1.83	1.81	0.0202	0.0168	0.0248	0.0273

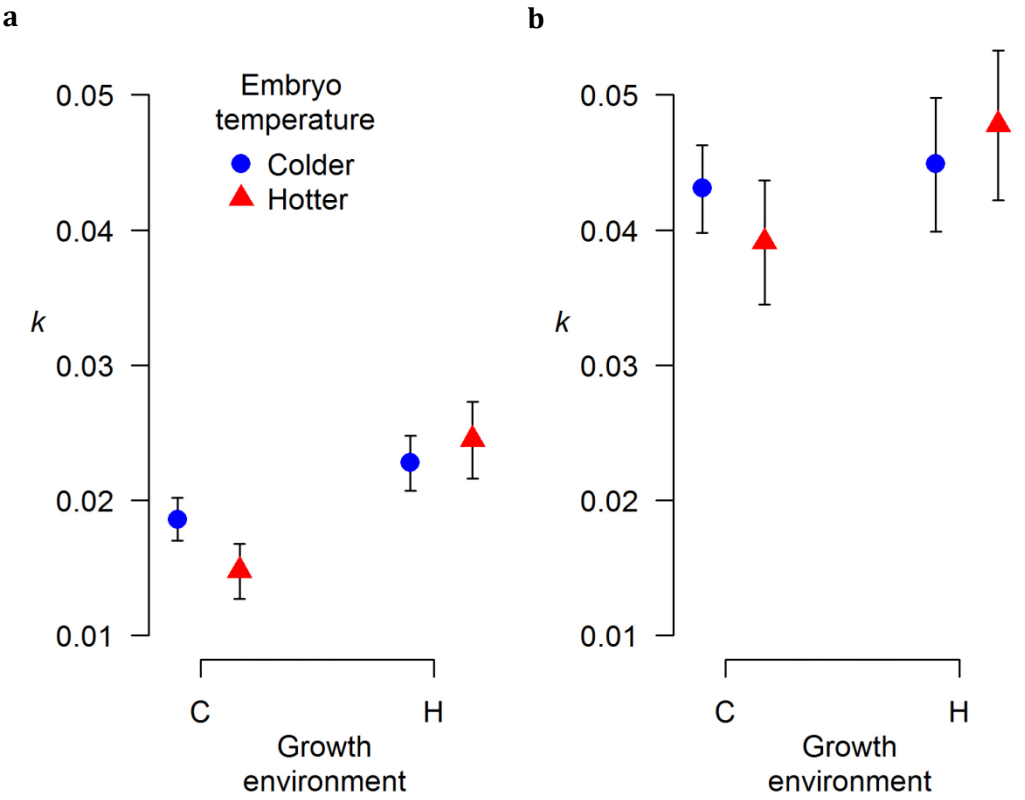

Figure 8: Bootstrapped *k* parameter estimates with 95% confidence intervals for female (a) and male (b) fish. C = colder and H = hotter growth temperature; blue circles = colder and red triangles = hotter embryo temperature.

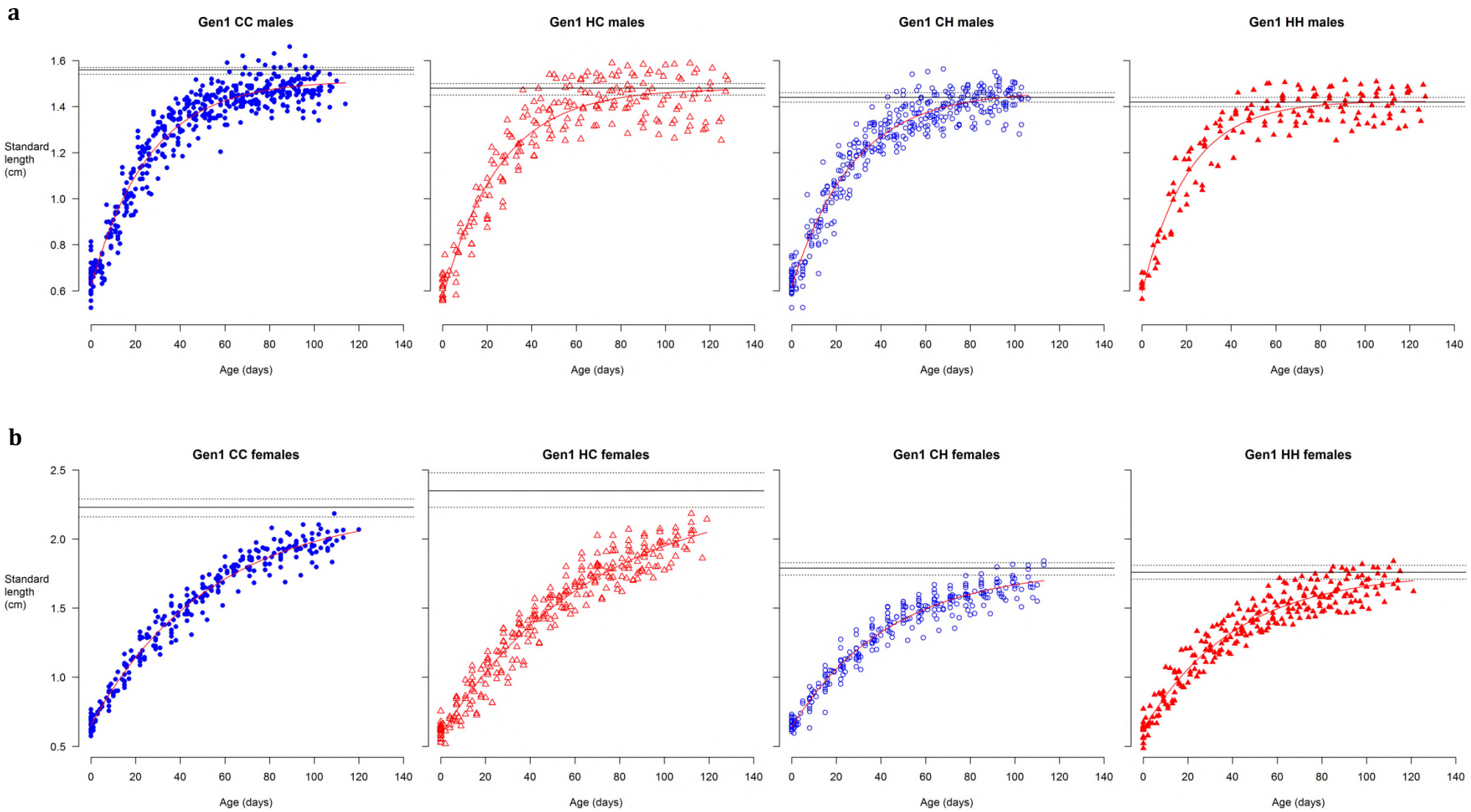

Figure 9: Growth curves of the male **(a)** and female **(b)** fish from each treatment in generation 1. Fish were measured every seven days and so the points are chronological measures of the same fish. The red line is the fitted Von Bertalanffy model; horizontal solid line is bootstrapped *Linf* estimate with 95% confidence intervals (dashed lines).

Part 3 – Generation 1 pregnancy characteristics and offspring size

Temperature had a strong effect of female size (see below) and consequently the size of mothers producing the offspring that were to become generation 2 differed **(Figure 10)**. The best fitting model explaining the observed differences in female size included embryo and growth temperature as well as the interaction between the two (adjusted $R^2 = 0.636$, $F(3,48) = 30.74$, p-value < 0.001). Coefficient estimates for the two predictors were embryonic temp (hotter), $\beta = -0.099$, $t = -2.442$, p-value < 0.05; growth temperature (hotter), $\beta = -0.322$, $t = -8.257$, p-value < 0.001; and the interaction between them, $\beta = 0.117$, $t = 2.043$, p-value < 0.05.

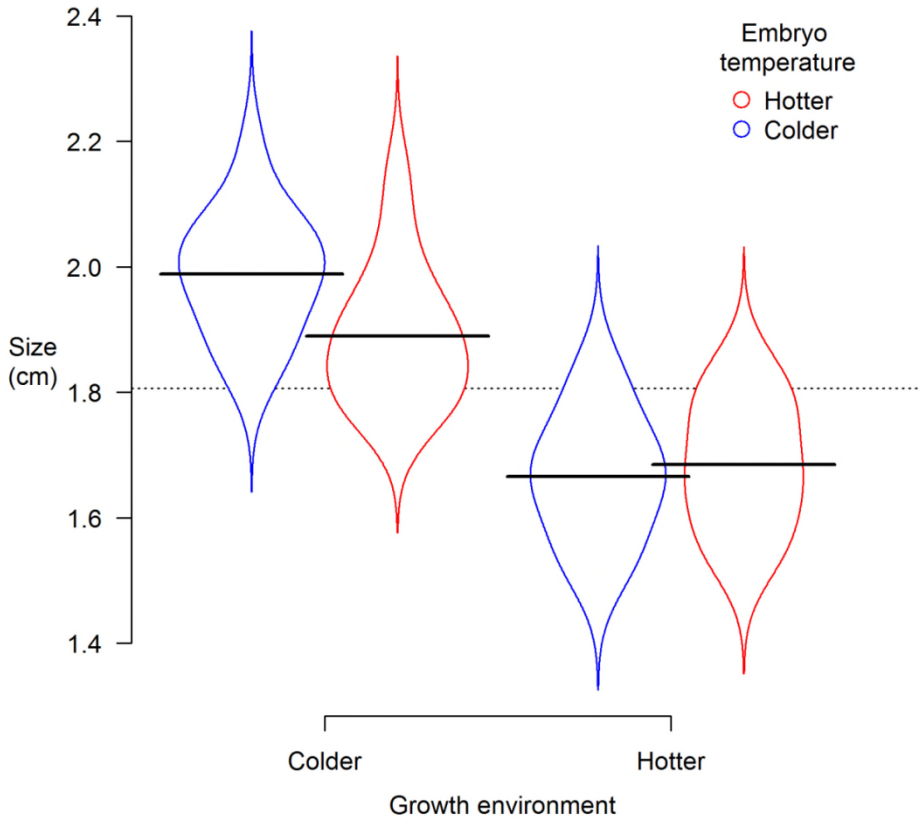

Figure 10: Sizes of females used to produce the offspring that became generation 2. Colder environmental temperature resulted in larger fish. There was an interaction between growth temperature and embryonic temperature indicated by hotter embryo fish being smaller than their colder embryonic counterparts in colder environmental conditions but larger than them in hotter environmental conditions.

There was no significant difference in gestation time between the four temperature regimes and similar to the founder generation there was a big range in gestation time **(Figure 11)**.

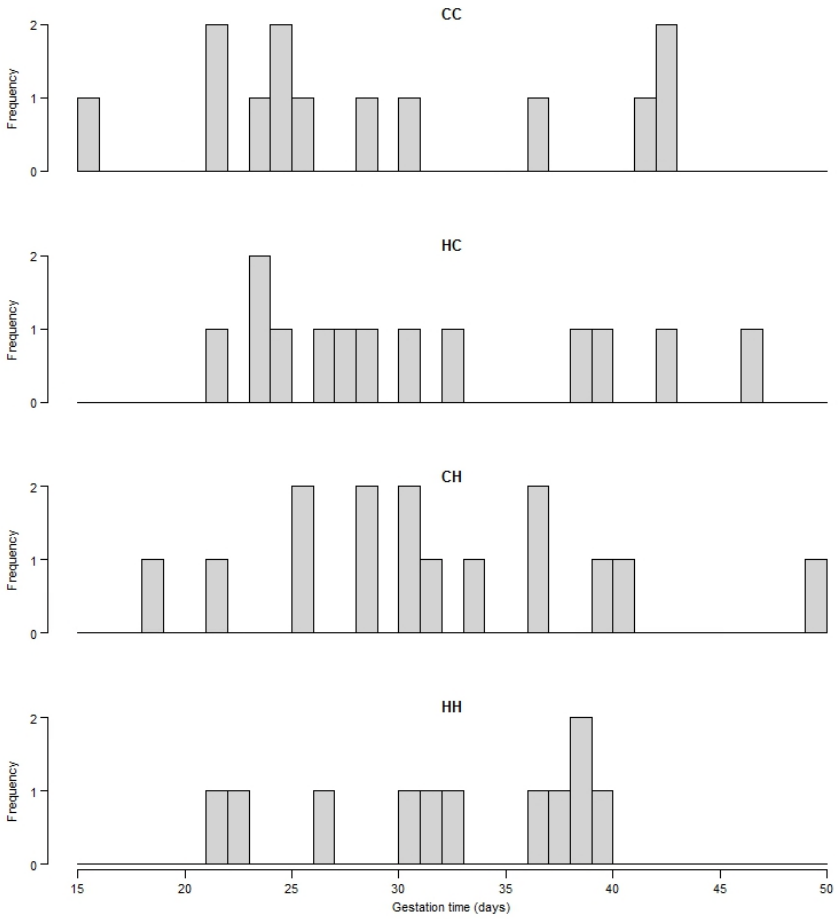

Figure 11: Histogram of gestation times for fish each of the four treatments. Again, there is no obvious pattern to indicate periodicity in reproductive cycles.

When accounting for female size, there was no clear effect of any of the four temperature regimes on SGR **(Table 6)**. Fecundity was higher in the colder environmental conditions but taking into account the mother's size (a result of environmental conditions), embryo temperature became a significant coefficient **(Figure 12 a & b)**. For the colder embryo conditions fecundity was described by the equation -18.42 + 12.45 * size (cm) whereas for hotter embryo conditions, fecundity = -21.972 +15.34 * size (cm), $R^2 = 0.853$, $F(4,48) = 69.65$, p-value < 0.001.

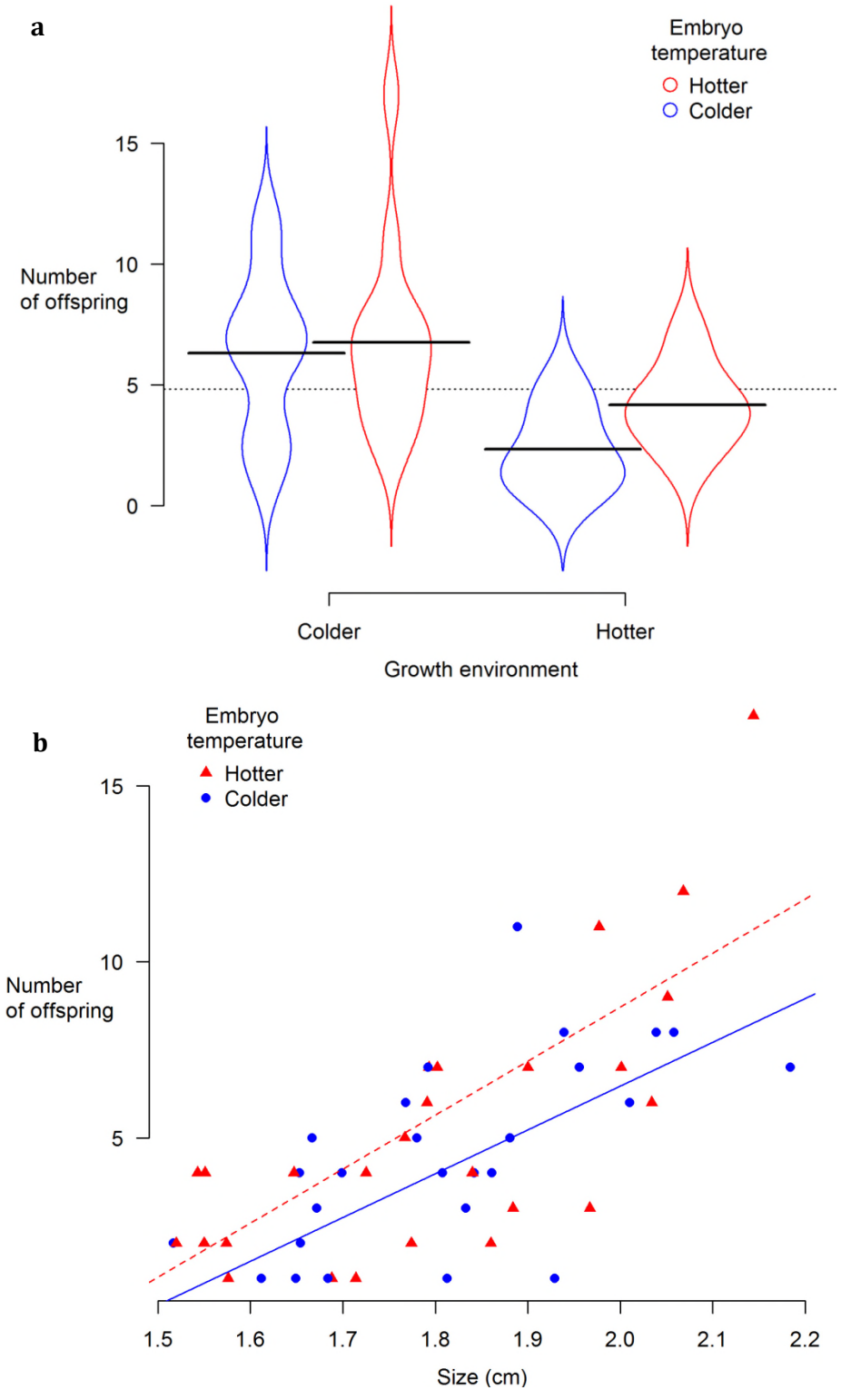

Figure 12: The effect of temperature and size on fecundity. **a)** The beanplot illustrates that fecundity was higher in the colder growth conditions. **b)** However, when accounting for female size embryonic temperature also affected fecundity with hotter embryonic conditions resulting in more offspring. Blue circles = colder and red triangles = hotter embryo temperature. Solid blue (colder) and dashed red (hotter) lines are linear regressions of fecundity against female size.

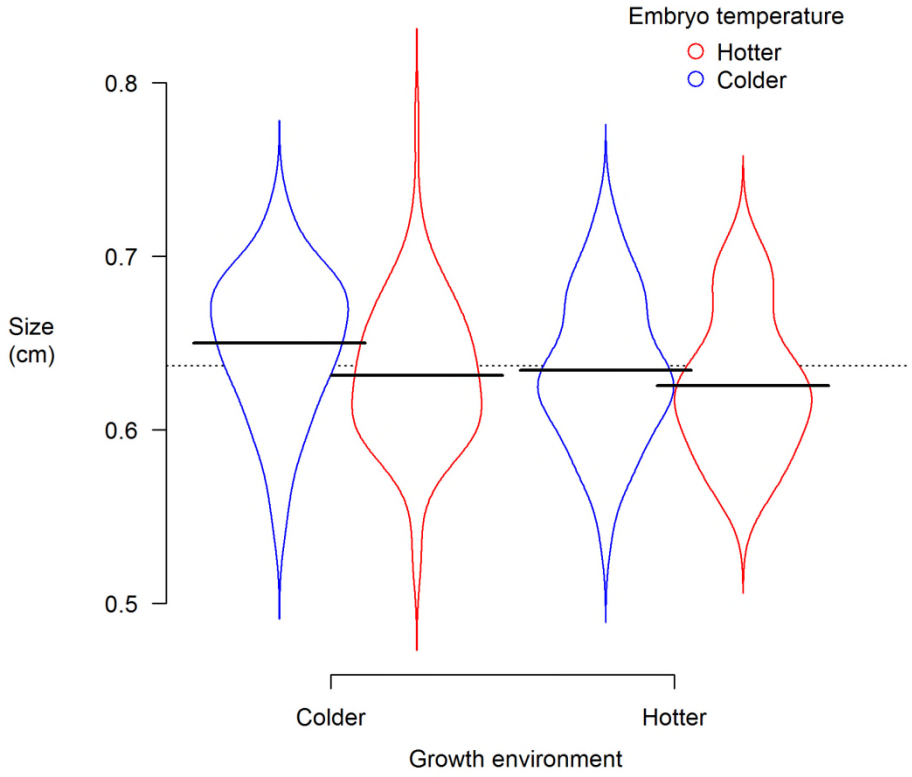

Figure 13: Beanplot of offspring size of fish born into each of the four treatments. Bean colour indicates embryo temperature and the x axis describes growth temperature. Offspring were largest in the colder embryo temperatures but only significantly different in the colder embryo-colder growth treatment.

Offspring size was significantly different between treatments (ANOVA $F_{3,246}$ = 4.511, p-value < 0.01) with post-hoc Tukey's HSD tests indicating the differences were between the CC and HH (p-value < 0.01) and CC and HC (p-value < 0.05) treatments **(Figure 13)**. Modelling the average offspring size for each female against temperature and female size did not result in such a clear distinction though. The model with the lowest AICc value included embryonic and growth temperature (adjusted R^2 = 0.106, F (2,48) = 3.957, p-value < 0.05) although the model which included female size only had almost equivalent support (adjusted R^2 = 0.078, F (1,49) = 5.235, p-value < 0.05) and an AICc difference of 0.25.

Table 6: Model coefficient estimates (with standard errors) from the 'best' models explaining the observed data. Best model was identified by lowest AICc value, na = not included in best model, ns = included but non significant in best model. The Adjusted R^2 value gives an approximation of the proportion of variance explained by the model, for count data (Fecundity) the adjusted D^2 is used.

		Founder			**Generation 1**		
		Estimate (se)	**Coefficient p-value**	**Model Adj R^2 (D^2)**	**Estimate (se)**	**Coefficient p-value**	**Model Adj R^2 (D^2)**
SGR	Intercept	1.238 (0.128)	< 0.001	0.066	0.333 (0.107)	< 0.01	0.069
	Environment (hotter)	-0.078 (0.025)	< 0.01		-0.031 (0.019)	ns	
	Female size	-0.586 (0.074)	< 0.001		-0.131 (0.055)	< 0.05	
Fecundity	Intercept	-1.074 (0.86)	ns	0.144	-3.949 (0.745)	< 0.001	0.558
	Embryo (hotter)	na			0.4 (0.128)	< 0.01	
	Female size	1.408 (0.491)	< 0.01		2.884 (0.386)	< 0.001	
Offspring size	Intercept	0.667 (0.008)	< 0.001	0.39	0.655 (0.007)	< 0.001	0.106
	Embryo (hotter)	-0.056 (0.012)	< 0.001		-0.016 (0.008)	< 0.05	
	Environment (hotter)	na			-0.017 (0.008)	< 0.1	

Part 4 – Generation 2 growth and adult size

Male and female fish were similarly sized at birth (t test t (166.194) = 0.92, p-value=0.356).

In order to investigate whether there were maternal effects, the full models in this generation started with the inclusion of growth, embryonic and mother's embryonic temperature.

As expected, growth was different between the two sexes with males growing to a smaller size (β = -0.793, t = -8.036, p-value < 0.001). Contrary to generation one, however, there was no interaction between sex and temperature suggesting that the "directional" effect of temperature is same for both sexes.

The best models describing *Linf* for both sexes included only growth temperature with hotter temperatures leading to smaller maximum size. For male fish the effect of temperature was only marginally significant (β = -0.045, t = -1.93, p-value < 0.1, adjusted R^2 = 0.033, F (1,80) = 3.726, p-value < 0.1), but was stronger for female fish (β = -0.135, t = -2.065, p-value < 0.05, adjusted R^2 = 0.035, F (1,88) = 4.266, p-value < 0.5).

K was different between the sexes (β = 0.022, t = 35.455, p-value < 0.001), with no interaction between sex and temperature, and males had a higher value of *k* than females. Embryonic temperature was the only explanatory variable retained in the best model for male fish (β = -0.005, t = -2.536, p-value < 0.05, adjusted R^2 = 0.063, F (1,80) = 6.429, p-value < 0.5). A model including the interaction between embryonic temperature and mother's embryonic temperature had similar support

(change in: R^2 = 0.012. F = 3.25, p-value = 0.015) although the simpler model has been reported here. There was no model that outperformed the null model for the *k* parameter in female fish.

Table 7: Growth characteristics of fish from each treatment in generation 2, values and 2.5 and 97.5% confidence intervals are the result of bootstrapping. *Linf* is the point at which fish stop growing and *k* is the Brody growth rate coefficient which is a measure of the speed at which fish reach *Linf*. The letter couplet indicates the *embryo* and *growth* temperature experienced in each treatment.

		Linf				***k***			
		CC	**HC**	**CH**	**HH**	**CC**	**HC**	**CH**	**HH**
		1.51	1.48	1.47	1.45	0.0385	0.0378	0.0368	0.0327
Males	2.5%	1.50	1.44	1.45	1.42	0.0361	0.0328	0.0341	0.0289
	97.5%	1.53	1.51	1.48	1.48	0.0409	0.0429	0.0394	0.0365
		2.29	2.26	2.17	2.13	0.0145	0.0144	0.0141	0.0147
Females	2.5%	2.22	2.18	2.11	2.06	0.0131	0.0128	0.0128	0.0131
	97.5%	2.36	2.35	2.23	2.21	0.0157	0.0159	0.0153	0.0164

The differences within treatment for *Linf* and *k* were much smaller than for the fish in generation 1 **(Table 7)**. *Linf* was slightly higher in the colder environments for both males and females but the difference was not as pronounced as in generation 1 **(Figure 14)**. *K* in male fish was slightly, but not significantly, higher in the cold environmental conditions and lowest in the HH treatment whereas for females, there was no difference in *k* between treatments **(Figure 15)**.

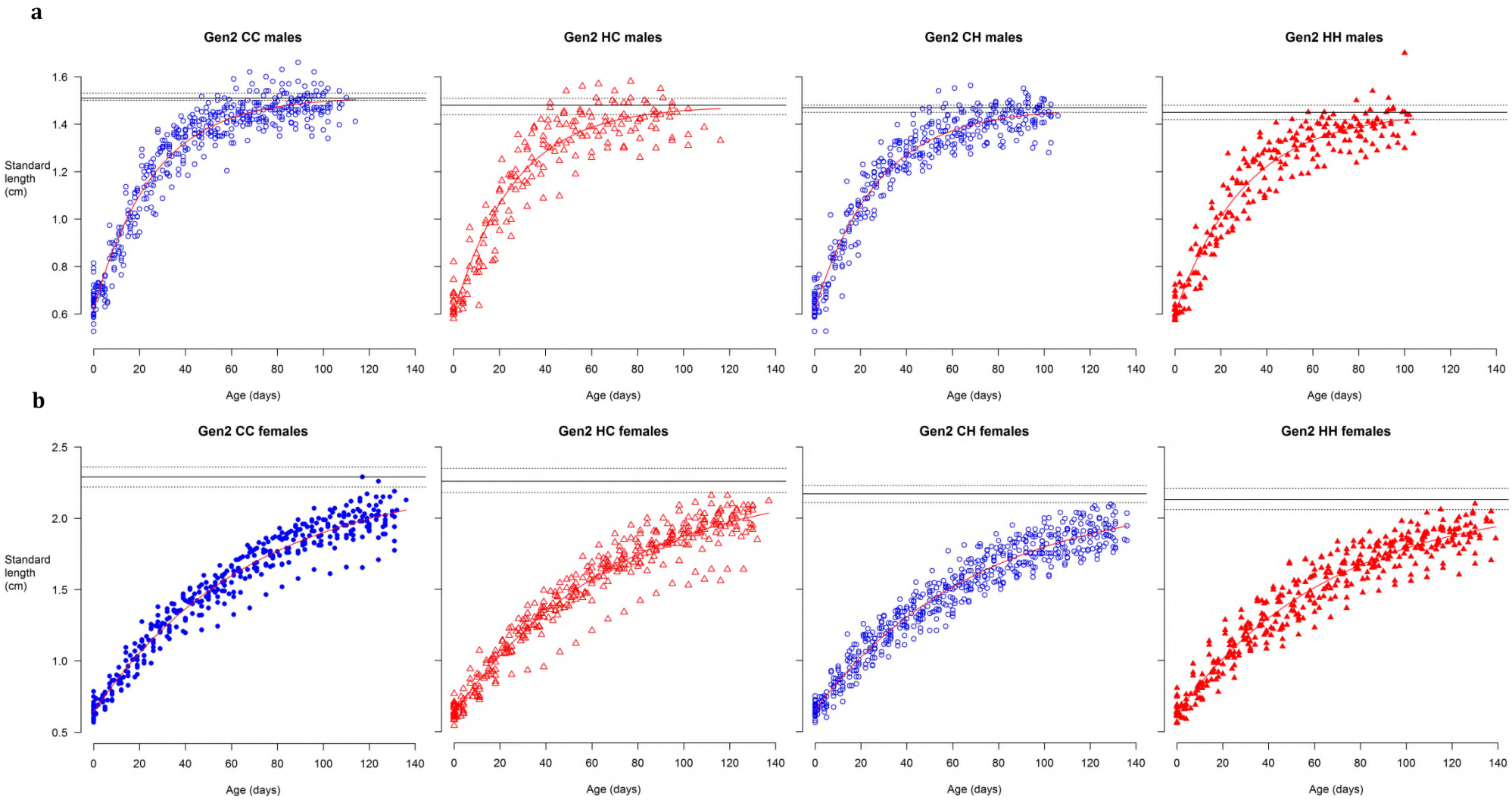

Figure 14: Growth curves of the male **(a)** and female **(b)** fish from each treatment in generation 2. Fish were measured every seven days and so the points are chronological measures of the same fish. The red line is the fitted Von Bertalanffy model; horizontal solid line is bootstrapped *Linf* estimate with 95% confidence intervals (dashed lines)

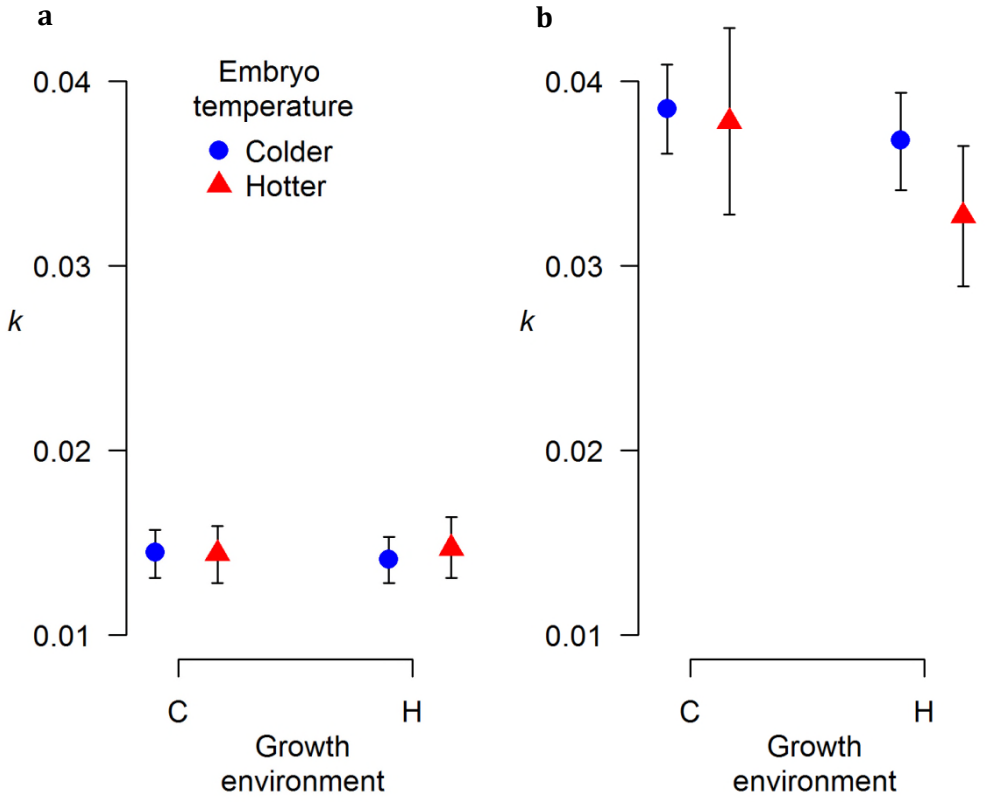

Figure 15: Bootstrapped *k* parameter estimate with 95% confidence intervals for generation 2 male **(a)** and female **(b)** fish. C = colder and H = hotter growth temperature; blue circles = colder and red triangles = hotter embryo temperature.

Discussion

The results from this series of experiments demonstrate that novel thermal regimes have an immediate and significant effect on offspring size and growth characteristics but that this effect is not as evident in the following generation. Furthermore, routine exposure to elevated temperatures which resulted in high levels of mortality when experienced continually **(Chapter 4)**, appear to be tolerable when experienced for short periods.

Part 1 and 2

The colder treatment resulted in larger offspring but without difference in fecundity or gestation time. Regardless of embryonic temperature females in the colder environmental conditions reached a larger adult size but at a slower rate than females in the hotter treatment. Males, conversely, reached a significantly larger maximum size in the *colder embryo-colder growth* (CC) treatment while each other treatment resulted in equivalent maximum adult size with similar growth coefficients in all four treatments. This part of the experiment simulates the immediate consequences for guppies entering a novel thermal regime (i.e. moving up/downstream in Trinidad or being released in a different location).

The results from the first two parts of this experiment support thermal biology life history predictions (Atkinson 1994, Angilletta 2009); colder temperatures resulted in larger offspring that attained a larger adult size while time until maturity – when significantly different – was shorter in hotter environments. There was no difference in gestation time, predicted to be shorter in hotter conditions, although there was a great deal of variability in gestation time

for both treatments masking any clear pattern. Guppy gestation time is often quite variable (Magurran 2005) and can be especially so for females on their first brood (Evans and Magurran 2000). Fecundity was similar across treatments and although theory predicts that larger offspring will result in reduced fecundity (Atkinson and Sibly 1997) there is evidence to suggest that lifetime reproductive output may be the cost paid for maintaining fecundity at lower temperatures (Fischer et al. 2006).

Part 3

The offspring born to the fish in the *colder embryo-colder growth* (CC) treatment were significantly larger than in any of the other treatments (which were similar in size to each other). This suggests that developing and growing in different temperatures has a long-lasting fitness consequence by affecting subsequent offspring size. Whether that is a function of the change in thermal regime or the exposure to the hotter thermal regime at either stage remains unclear. Female guppies are capable of manipulating offspring characteristics depending on environmental or competitive conditions (e.g. Bashey 2006) but temperature will also have direct effects on development time (Atkinson 1996) and it appears that the offspring size in this generation resulted from a combination of maternal investment and temperature.

Fecundity was higher in the colder environmental conditions. As fecundity depends on female size and females in the colder environment grew to a larger size more offspring were produced in the colder treatment. When adjusting for the size differences, however, fecundity was relatively higher in the hotter conditions. The

growth characteristics of these fish indicated no transgenerational effect of their mother's embryonic temperature and there was no significant difference in maximum size or growth between any of the treatments for either males or females.

Part 4

Growth in generation two was comparable across treatments and had similar predicted maximum sizes to the largest fish in generation one. The growth coefficient, however, was lower in all treatments compared to the first generation indicating that fish in generation two were taking longer to reach maturity and their maximum size. Only male fish in the *hotter embryo-colder growth* (HC) treatment had a comparable *Linf* and *k* values across successive generations. In each of the other treatments *k* was much lower in generation 2 while *Linf* was equivalent except for females in the hotter treatment which increased to the same as the colder treatments.

The duration of development and timing of physiological events has lifetime consequences (Spicer and Burggren 2003, Spicer et al. 2011) and both are sensitive to temperature. In my experiment, however, growth parameters seemed to be mostly affected by the growing environment rather than developmental effects. Previous work has suggested that fluctuating temperatures have significant developmental effects when they are approaching the physiological thermal limits of the organism in question (Arrighi et al. 2013). Consequently, the factor which caused similarity between treatments may be the characteristics of the thermal regimes rather than solely the extreme values. The range and upper and lower

temperature values of each regime were unquestionably dissimilar however the mean and median temperature for the two treatments differed by only ~2^{0}C (hotter mean/median = 27.4/27.2^{0}C, colder mean/median =25/25.2^{0}C).

In a separate long-term study Breckels et al. (2013) exposed guppies to different thermal regimes (25^{0}C or 28^{0}C) for 24 months (~8 generations) and, although there was a large reduction in genetic diversity, observed no change in male length, size at maturity or population size. It is possible that the mean/median temperature difference between thermal treatments in this experiment (which are similar to those of Breckels et al.) was too small to result in significant differences between them and suggests that the strength of effect may be proportional to the duration of exposure to each temperature. It is also possible that I attempted to record fitness characteristics on too short a time scale and that long term measures of fitness (e.g. lifetime fecundity, lifespan) would identify fitness consequences associated with either regime. Even so, this still does not adequately explain the immediate differences in offspring size and growth I recorded in the first generation.

When exposed to 30^{0}C for 70 days Muñoz et al. (2012) demonstrated that the guppy's resting metabolic rate (RMR) became elevated and they were unable to acclimate their metabolic rate to high temperatures. Higher temperatures result in increased oxygen metabolism and therefore production of reactive oxygen species (ROS) (Metcalfe and Alonso-Alvarez 2010, Hemmer-Brepson et al. 2014). Furthermore, mitochondrial decoupling which results in less efficient mitochondria is also increased at higher temperatures (Abele et al. 2002). The

efficiency of mitochondrial phosphorylation is positively correlated with larger body size (Salin et al. 2012). Therefore, based on the assumption that a larger offspring and adult size has greater fitness (Kingsolver and Pfennig 2004), as the guppies in this experiment demonstrated comparable growth across all treatments one expects some kind of trade-off or compensatory effect to account for this imbalance (Stearns 1989). The alternative hypothesis to this, however, is that as male size did not vary much between treatments or across generations it could be that adult size is canalised and therefore inflexible over such a short time period (Gibson and Wagner 2000).

Compensatory or catch-up growth is an acceleration in growth rate, generally fuelled by higher food intake, so that the effects of environmental stressors in early life have a minimal impact on final body size (Ali et al. 2003, Mangel and Munch 2005). The increase in growth rate comes with multiple and varied costs physiologically affecting immunity, digestive efficiency, metabolism and behaviourally through increased risk of predation and reduced time spent reproducing resulting from a greater portion of time spent foraging (Dmitriew 2011). Furthermore, higher growth efficiency is metabolically more costly and will cause more energy to be diverted from maintenance or reproductive functions (see Orizaola et al. 2014 and references therein). Oxidative stress resulting from higher temperatures or compensatory growth has been demonstrated to affect pigments/melatonin production (Metcalfe and Alonso-Alvarez 2010) while reproductive characteristics such as sperm traits and reproductive motivation may be the "Achilles heel" of tropical ectotherms (Zeh et al. 2012). These would be important traits to investigate when attempting to explain the similarity in growth

rates between treatments in this experiment. Consequently, in **Chapter 6** I investigate the effect of novel thermal regimes on colour, sperm and mating behaviour.

This experiment has demonstrated that exposure to novel thermal regimes has a strong and immediate effect on guppy life history traits and yet by the following generation those effects had largely disappeared. Further investigation into reproductive life history characteristics is necessary to try and identify the cost of this response although the cost is evidently not great enough to restrict reproductive success in the early stages of environmental change.

Chapter six: The effect of thermal regime on sexually selected traits

: hotter conditions cause a change in black & orange colouration but not sperm size or mating behaviour… #MixedSexualSignals

Lay summary

The previous chapter indicated that male growth does not vary greatly in response to temperature. In the absence of a strong effect on growth I investigated whether thermal regime had an effect on three important reproductive characteristics: body colouration, mating behaviour and individual sperm size. In guppies, increased colouration has been suggested as an 'honest' signal indicating the quality of a male's sperm. Similarly, male guppies with high sperm loads tend to perform more sigmoid displays than sneak mating attempts. If male size at maturity is fixed, yet elevated temperatures are energetically costly I expected males from the hotter treatment to have less energy to allocate to reproductive traits. However, the amount of orange colouration was higher in hotter environmental conditions while male mating behaviour and sperm length was similar between the two thermal regimes. The results indicate that when faced with new conditions male guppies remain capable of attracting and reproducing with a mate to sire a new generation. They further reinforce our ideas that guppies are broad thermogeneralists and are relatively unaffected by exposure to novel thermal regimes – at least during the time window from birth to maturity studied in this experiment. This study raises the question as to whether there is subsequently a longer term consequence of changing environmental conditions such as a reduction in lifetime fecundity or shorter lifespan.

Abstract

The absence of significant differences in patterns of male growth across different thermal regimes may suggest a trade-off which maintains growth at the expense of another characteristic. On reaching maturity guppies cease growing and invest all of their energy into reproduction and systems maintenance. Consequently, to identify the trade-off I focussed on three reproductive characteristics: body colouration, mating behaviour and individual sperm size. Male guppies court females with colourful displays and a larger area of colour indicates males are of higher quality. Furthermore, males with higher sperm loads spend more time displaying to females. As elevated temperatures are more energetically costly I predicted that colouration, sperm size and mating behaviours would be lower in the hotter treatment. As in previous experiments mating behaviour was resilient to temperature and there were no differences observed between the two regimes. Sperm length decreased in the hotter treatment in the first generation although by the second generation there were no differences in length. While accounting for differences in body size, orange colouration was greater in hotter conditions which may signal an increase in antioxidant defences. The results from this experiment further demonstrate the guppy's ability as a thermal generalist. They also suggest that in the early stages of environmental change male guppies remain capable of fathering offspring and thus ensuring their short term survival and providing more time to adjust to the novel conditions.

Keywords

Variable temperature – mating behaviour – colouration – sperm length – secondary sexual characteristics

Introduction

The effect of increasing temperature negatively impacting ectotherms' reproductive fitness has been suggested as one of the most damaging consequences of climate change (Zeh et al. 2012). Temperature exposure at all life stages has profound direct and indirect effects on the reproductive success of an organism. It is therefore of great importance to understand the effects of temperature on sexual traits due to their fundamental importance to overall fitness.

The temperature experienced during developmental stages has both immediate and lifetime consequences by affecting egg/offspring size (Atkinson 1994), clutch size/fecundity (Smith and Fretwell 1974), development time (Spicer et al. 2011), interbrood interval (Collin 2012) and potential final size (Garcia de la serrana et al. 2012). Post birth, the temperature experienced by fish will affect growth as well as age and size at maturity (Kuparinen et al. 2011) and have direct effects on reproductive tissues (Dorts et al. 2012). A major influence on the maturation of gonadal tissues is the effect of temperature on sex steroids which in turn impacts reproductive characters such as spawning efficiency, ovulation, oocyte size, fecundity, fertilisation rate (Arantes et al. 2011) as well as oogenesis and spermatogenesis (Dorts et al. 2012). Furthermore, temperature can have powerful impacts over short exposure (< 8 days) affecting spawning activity and causing a reduction in estradiol in females and testosterone in males (Soria et al. 2008). The phenological effects of temperature altering spawning and gestation time are of particular importance in seasonal environments where the time

available for growth and reproduction are limited to seasonal windows although shorter developmental time can result in shorter generation times thus improving fitness regardless of seasonal effects (see Dmitriew 2011).

Once mature, the fundamental effect of environmental temperature continues to influence reproductive success whether by altering swimming performance (Wilson 2005), time budgets (Valdimarsson and Metcalfe 1999, Rensing and Ruoff 2002), resource allocation (Valencak et al. 2009), aggression (Biro et al. 2010) or predator avoidance (Weetman et al. 1998, 1999). For species subject to environmental change or released into new environments how they respond to the multitudinous effects of temperature in the first few generations will determine their success or failure at persisting and then establishing in the new conditions. Integral to surviving is the ability to attract mates and reproduce successfully.

In this experiment I looked at the effect of temperature on the mating behaviour, sexual ornamentation and sperm size of successive generations of male guppies exposed to a novel thermal regime.

Post maturation, male guppies grow very little and invest all their energy into reproduction and maintenance. There is a fitness benefit in attaining a larger size – e.g. competitive and reproductive success, predator avoidance – however secondary sexual traits such as area of colouration are important independently of total size as they too provide an indication of the quality of the mate on offer (Houde 1997). Virgin female guppies usually express interest in mating although once mated for the first time females are able to store sperm and are generally

disinterested in male attention from then on (Liley 1966). Furthermore as females are polyandrous and can exhibit strong mate choice (Evans and Magurran 2000, Pitcher et al. 2003) there is selective pressure on male guppies to either develop strong secondary sexual traits or make behavioural changes to mating tactics depending on the current conditions (e.g. Reynolds et al. 1993) and social situations (Magellan et al. 2005). A greater rate of displays behaviour has been associated with female preference (Bischoff et al. 1985), is a good measure of the male's physical condition (Nicoletto 1996) and is positively correlated with sperm production (Matthews et al. 1997, Evans et al. 2002). Sneak mating, conversely, is non-consensual and transfers fewer sperm but it is a less energetically costly, while still effective, mating tactic (Houde 1997, Matthews and Magurran 2000).

Sexual ornamentation is a signal of a mate's overall quality (von Schantz et al. 1999) and potentially the likelihood of his sperm's fertilisation success (Blount et al. 2002). Male guppies with larger tails (Bischoff et al 1985) and increased area (and intensity) of orange colouration are more attractive to females (Houde 1987, Kodric-Brown 1989, Brooks and Caithness 1995). Colouration is seen as an honest indicator of male quality and is linked with foraging ability (Grether et al. 2001a); better swimming performance (Nicoletto 1991, 1993); faster and more viable sperm (Locatello et al. 2006); and greater ejaculate size (Pitcher et al. 2007). Increasing temperature results in greater oxidative stress (Metcalfe and Alonso-Alvarez 2010). There is debate, however, as to whether increases in carotenoids or melanin based pigments are signals of an ability to withstand oxidative stress (reviewed in McGraw 2005, Perez-Rodriguez 2009) or are promoted by because antioxidants are required in their synthesis (Galvan and Alonso-Alvarez 2008).

Sperm morphology, across taxa, is closely associated with fertilisation success (Immler et al. 2010) however for guppies the link with fitness sperm length is unclear. Increased area of colour has been correlated with longer sperm in some studies (e.g. Pitcher et al. 2007) while no relationship was observed by (Locatello et al. 2006) and male size cited as responsible for longer sperm in another (Skinner and Watt 2007). Male age is a further factor affecting sperm length with an increase in longer – but slower – sperm in older males although there was no difference in fertilization success between ages (Gasparini et al. 2010). The effect of temperature also has varied and sometimes contradictory effects on sperm length. Based on the temperature experienced from birth Breckels and Neff (2013) observed that sperm length was shorter at the higher temperature. However in a separate study, while sperm length decreased over successive generations the fish maintained at higher temperatures had consistently longer sperm than fish maintained at lower temperatures (Breckels and Neff 2014).

The results from the previous chapter demonstrated that temperature has the potential to alter size and growth characteristics and that guppies are able to exhibit similar growth characteristics despite differing thermal regimes. My aim in this series of experiments was to determine if the guppy's ability to maintain similar growth trajectories came at the cost to sexually selected traits such as: mating behaviour, quantity of orange and black pigmentation and sperm morphology. Consequently my question in this experiment was:

What are the effects of environmentally-realistic fluctuating temperatures on sexually selected traits?

Taking into account fish size, I predicted that the colder thermal treatment would have a positive effect on the area of colour and sperm length of each fish. The colder treatment is less metabolically costly thus allowing more resources to be allocated to these traits. Mating behaviours have proven resilient to environmental temperature after short **(Chapter 3)** and prolonged **(Chapter 4)** exposure I expected them to be similar between treatments. The results from these experiments should provide an indication on how fluctuating temperatures affect important reproductive fitness traits.

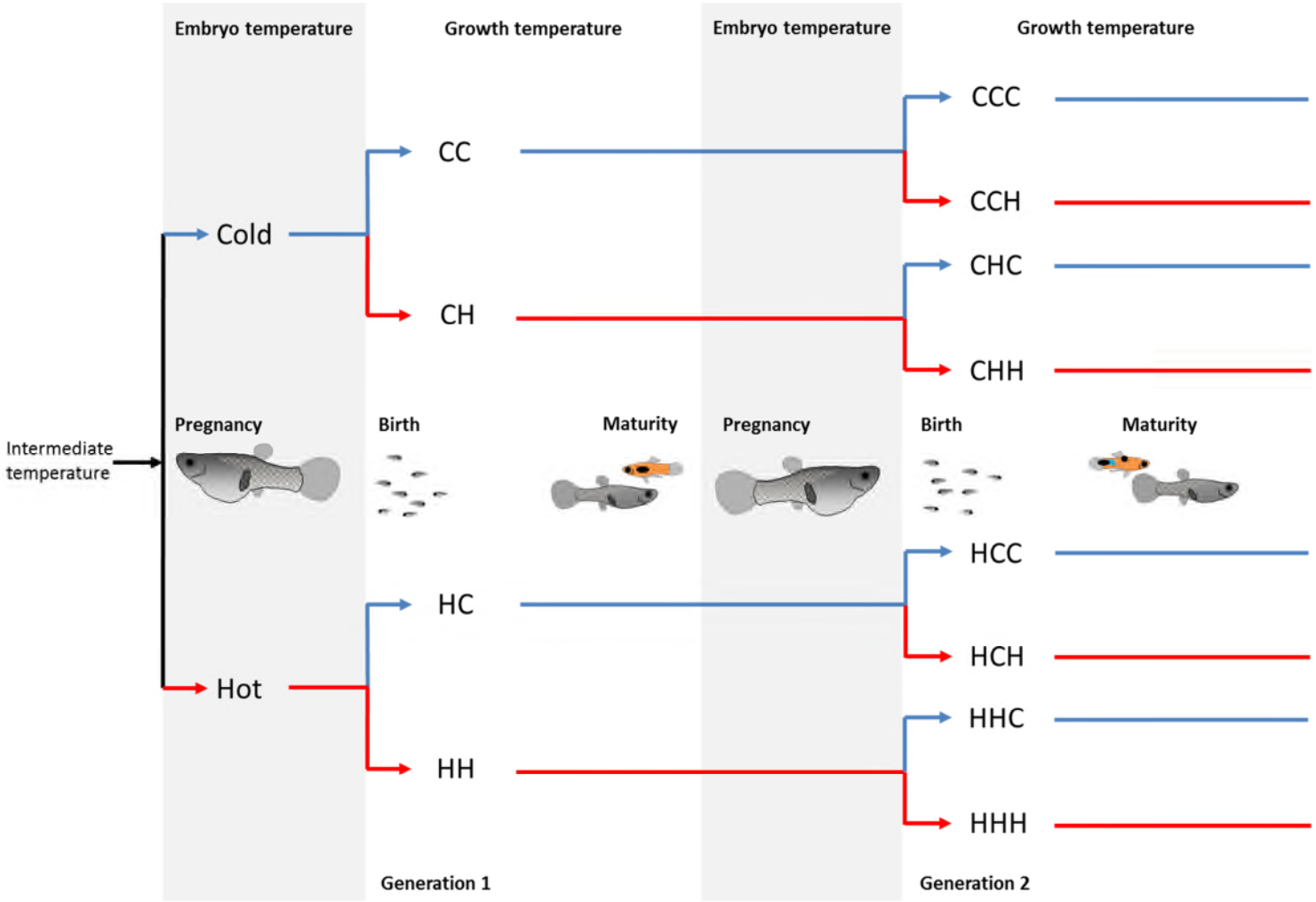

Figure 1: Experimental design. Virgin females from our lab aquarium were mated and placed into one of two thermal regimes until they gave birth whereupon they were retired from the experiment. These offspring were split equally between the thermal regimes then photographed and measured each week until maturity. One mature, females were mated with males from the same treatment and then returned to their individual container until giving birth. These offspring were then split between thermal regime and measured weekly until maturity. Behaviour, colour and sperm analyses were performed when all males had matured in each generation.

Methods

The fish used in these experiments came from the experimental system descried in C**hapter five** and **Figure 1**.

Colour analysis

Males were terminally anaesthetised with an overdose of MS222 (Tricaine methanesulfonate). Orange colour is permanently displayed and, as black colouration is under partial neurological control, after death accurate measurements of these colours are possible (Houde 1997). Areas of orange and black are similar on each side of guppies with asymmetry uncommon (Sheridan and Pomiankowski 1997). Consequently, males were photographed on their left hand side with a measurement scale and the area and number of orange and black spots were measured using *ImageJ* **(Table 1)**.

Measurement error was estimated by repeatedly measuring colour characteristics of three fish and then determining the Intraclass Correlation Coefficients (ICC) using the ICC package in R (Wolak et al. 2012). ICCs for all variables (body area, length, area of orange and black) were all in agreement with over 95% confidence.

Mating behaviour

A single male guppy was released into a 45 litre tank containing two females and allowed to settle for 10 minutes. During the following 10 minutes the male's sigmoid displays and sneak mating attempts were recorded. Two females were used to dilute the amount of attention the male guppy, on his first exposure to a

female, divested on one animal. This protocol was repeated for male fish from generation two **(Table1)**. The pairing of females was randomised throughout the observations to minimise the likelihood of the same pairing of females being used twice (gen 1 females n = 24, range (cm) = 1.66-2.68, sd = 0.239, gen 2 females n = 36, range (cm) = 1.75-2.38, sd = 0.17).

Sperm length

After photographing an individual, a few drops of phosphate buffered saline (PBS) were placed on the male and following the method of Matthews 1997 and Evans et al 2003, sperm bundles (zeugmata) were extracted by swinging the gonopodium forward and applying light pressure to the abdomen at the base of the gonopodium.

A 100ul sample of PBS containing multiple zeugmata was placed in a separate microcentrifuge tube and gently vortexted before adding 5ul of formalin to fix the individual sperm. This was repeated 3 times for each individual.

5ul of fixed sperm solution was placed onto a standard microscope slide with a coverslip and viewed under a microscope at 100x magnification using oil immersion and phase contrast. Images of individual sperm were taken using a digital camera (Olympus SP500uz) which were then measured using ImageJ. The head and midpiece plus flagellum length (ul) of ~20 sperms per individual were measured (gen 1 n=660 spermatozoa measured from 33 males, gen 2 n=1335 spermatozoa measured from 73 males) and the average value for each individual used in the analysis **(Table 1)**.

Table 1: Numbers of males from each treatment used in behaviour, colour and sperm analyses in both generation 1 and 2. Within-gen treatment represents the temperatures males experienced as an embryo and during growth; cross-gen treatment includes the embryonic temperature experienced by a male's mother. In generation 1 the letter couplet indicates the *embryo* and *growth* temperature experienced in each treatment, in generation two this couplet is preceded by the letter indicating their mother's embryonic temperature.

	Generation 1		**Generation 2**		
	Treatment	Number of males	No. males (within-gen treatment)	Treatment (cross-gen)	No. males (cross-gen treatment)
Colour patterns	HH	7	15	HHH	7
				CHH	8
	HC	12	11	HHC	7
				CHC	4
	CH	12	23	HCH	16
				CCH	7
	CC	11	27	HCC	14
				CCC	13
Mating behaviour	HH	7	15	HHH	7
				HHC	8
	HC	12	12	HCH	7
				HCC	5
	CH	13	23	CHH	16
				CHC	7
	CC	14	29	CCH	16
				CCC	13
Sperm length	HH	7	14	HHH	6
				CHH	8
	HC	11	9	HHC	6
				CHC	3
	CH	6	19	HCH	13
				CCH	6
	CC	8	24	HCC	12
				CCC	12

Analysis

The effect of embryonic and growth temperature on each dependent variable was analysed using a 2-way ANOVA in R. A 3-way ANOVA was used to assess the effect of maternal thermal experience with the inclusion of maternal embryonic temperature. Standard length (sl) and age were included as covariates in the sperm length models, sl and female standard length was included in the behavioural models and male body area was included in modelling colour traits to account differences in between fish during model selection.

Model selection was made by minimising AIC (Akaike Information Criterion) values (Akaike 1973, Symonds and Moussalli 2010). Due to the small sample sizes, a derivative of AIC – AICc – was used to compare the models as it has a built in small sample bias correction (Johnson and Omland 2004). Count data was modelled using a generalised linear model (glm) with a poisson distribution. As count data is quite frequently overdispersed, the Quasi-AIC (QAIC) value, which takes overdispersion in the form of a variance inflation factor into account, was employed in the same way as AIC where necessary (Burnham and Anderson, 2001, Richards 2008). The D^2 (a measure of the deviance explained by each model) and adjusted D^2 (which can be used to compare models with different variables and interactions) was calculated according to the equations and theory detailed in **Chapter 3**.

Results – Colour

Table 2: Colour characteristics (and standard deviation) of fish in each of the four treatments. Area of black and orange colour was measured from photographs of the left hand side of fish. Total colour is the sum of the area of black and orange colour. The letter couplet indicates the *embryo* and *growth* temperature experienced in each treatment.

	Generation 1				**Generation 2**			
	CC	**HC**	**CH**	**HH**	**CC**	**HC**	**CH**	**HH**
Colour replicates	11	12	12	7	27	11	23	15
Total colour (mm^2)	0.48	0.61	0.5	0.48	0.59	0.55	0.72	0.62
sd	0.13	0.22	0.2	0.15	0.198	0.077	0.21	0.153
Total orange (mm^2)	0.37	0.48	0.42	0.4	0.48	0.42	0.06	0.51
sd	0.14	0.22	0.19	0.14	0.19	0.006	0.02	0.17
Total black (mm^2)	0.111	0.123	0.082	0.08	0.12	0.13	0.12	0.12
sd	0.04	0.037	0.016	0.033	0.032	0.039	0.056	0.055
No. orange spots	2.9	3.7	2.4	2.4	2.6	2.7	2.7	2.3
sd	0.64	0.71	0.55	0.79	0.99	0.71	0.67	0.75
No. black spots	1.5	2	1.8	1.6	2	1.9	2.1	1.8
sd	0.53	0.5	0.84	0.79	0.52	0.33	0.62	0.44
Mean orange/spot (mm^2)	0.13	0.13	0.17	0.018	0.02	0.016	0.023	0.023
sd	0.055	0.054	0.067	0.0079	0.0086	0.0036	0.0075	0.0081
Mean black/spot (mm^2)	0.076	0.063	0.053	0.054	0.061	0.069	0.059	0.064
sd	0.018	0.018	0.024	0.014	0.014	0.02	0.025	0.024

Generation 1:

Total colour per fish (the combined area of black and orange colour on one side) was not significantly different between treatment in generation 1 (ANOVA $F_{3, 38}$ = 0.131, p-value = 0.941). Total orange was similar across the four treatments and no

differences were explained with the models, however total black was higher in the colder environments **(Table 2, Figure 2)**. While accounting for body size (β = 0.025, t = 1.521, p-value = 0.137) and age (β = 0.00008, t = 1.706, p-value < 0.1); growth temperature (β = -0.0021, t = -1.516, p-value = 0.137) was included – although not as a significant coefficient – in the best model explaining area of black colour (adjusted R^2 = 0.131, F (3, 38) = 3.062, p-value < 0.05).

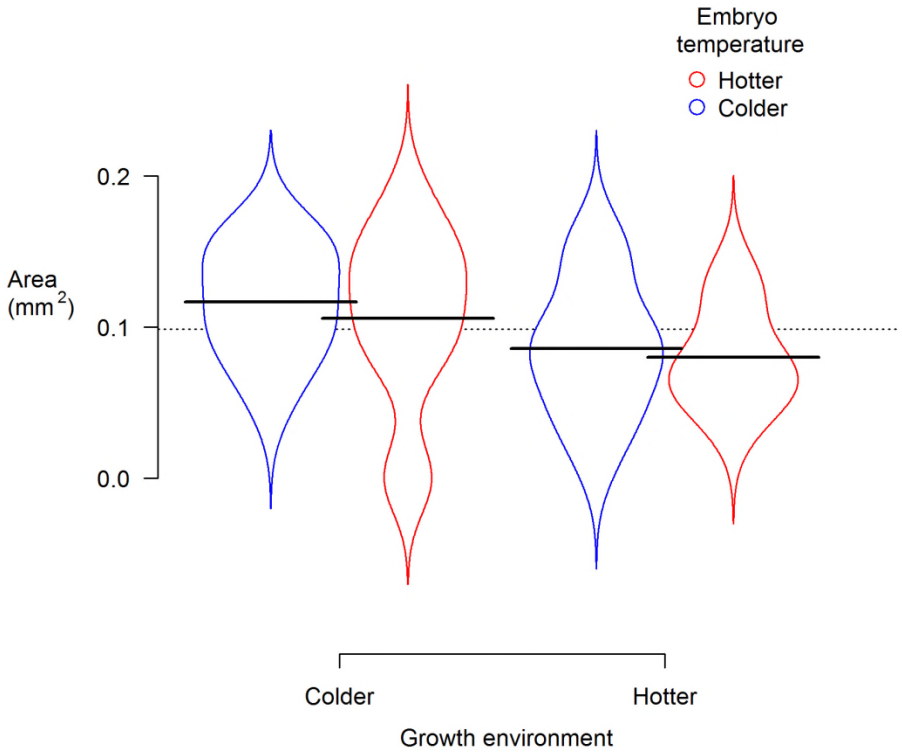

Figure 2: Beanplot of total area of black on fish in each treatment, colder growth environments led to greater black colouration.

There was no difference in the number of spots (chi-square (black) = 10.42, df = 9, p-value = 0.318, chi-square (orange) = 19.12, df = 12, p-value = 0.08) between treatments however mean spot size did vary **(Figure 3)**. The models, again taking into account body size and age, which best described both mean orange and mean black spot size both included growth temperature although with opposite effects. Orange spot size was larger (β = 0.0046, t = 2.223, p-value < 0.05)

while black spot size was smaller (β = -0.0011, t = -1.76, p-value < 0.1) in the hotter environment.

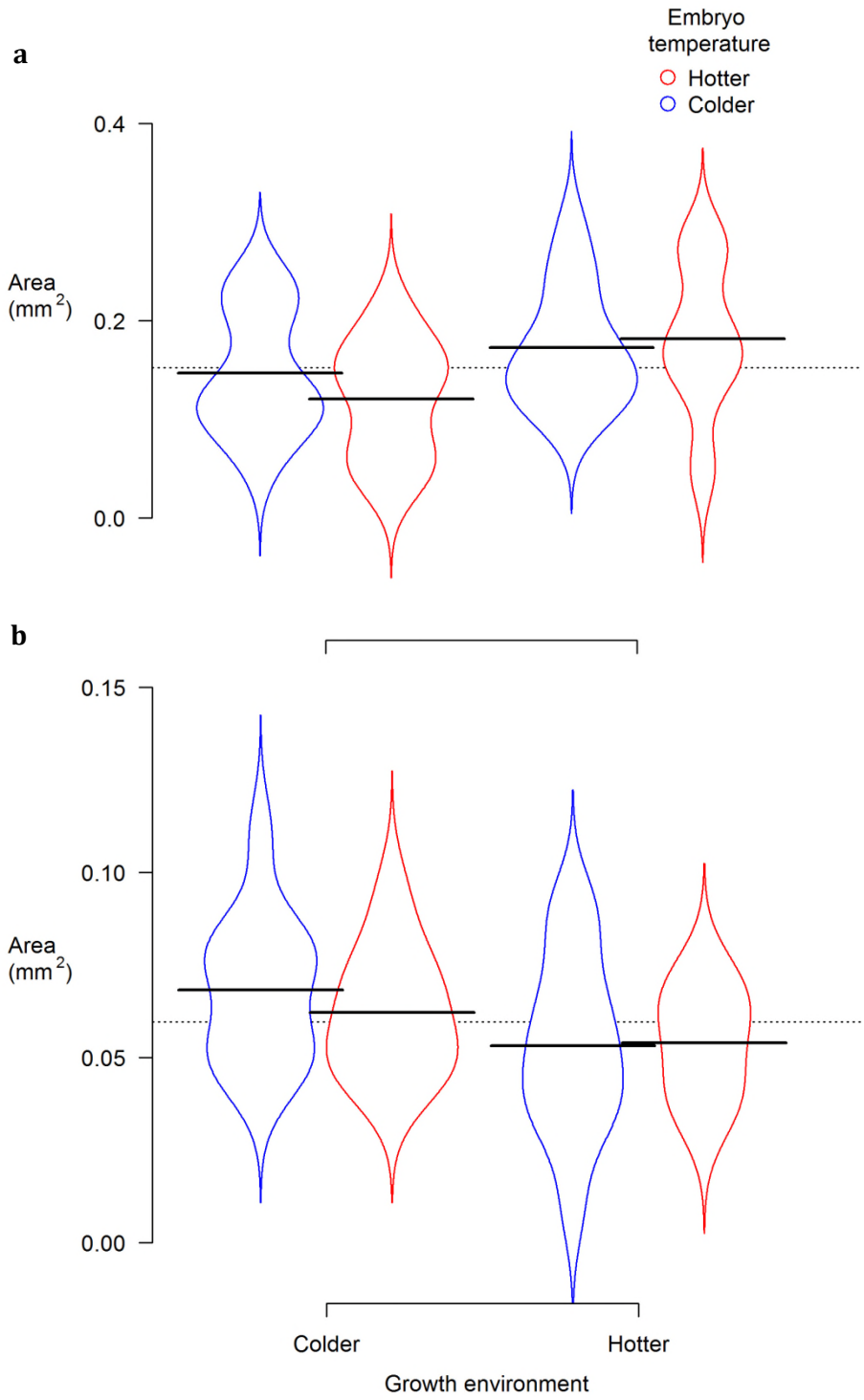

Figure 3: Mean orange **(a)** and black **(b)** spot size across treatments. Orange spots were larger and black spots smaller in hotter environments.

Generation 2:

Taking the size of the fish into account (β = 0.129, t = 1.997, p-value < 0.05), growth temperature had a strong effect (β = 0.012, t = 2.706, p-value = 0.01) on the total colour of each male with males from the hotter environmental conditions having more colour (adjusted R^2 = 0.079, F (2, 73) = 4.23, p-value < 0.05, **Figure 4**).

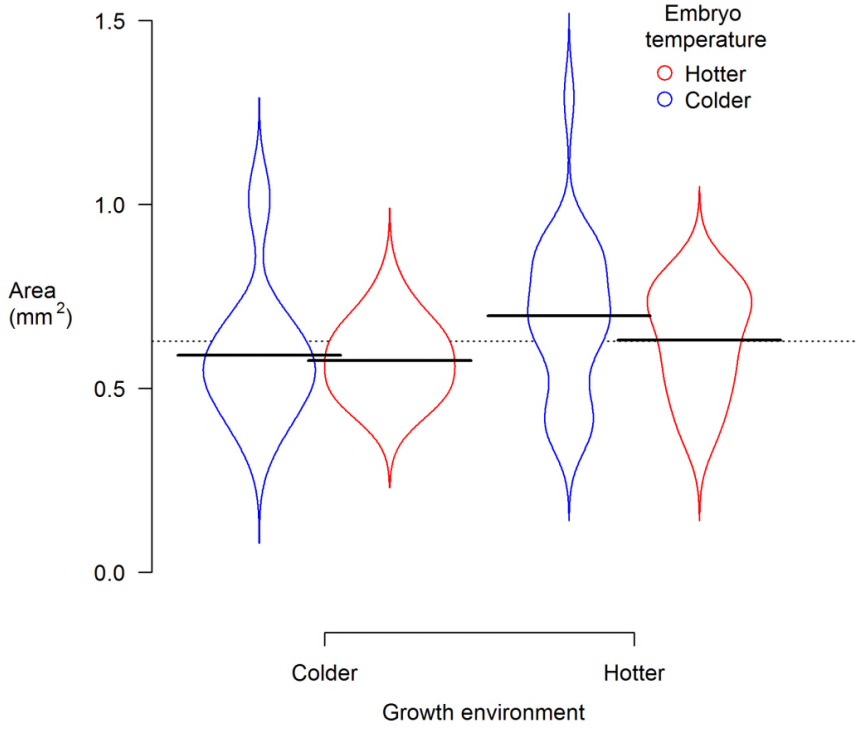

Figure 4: Differences in total colour between fish in each treatment, Hotter growth conditions led to greater total colour.

The best model explaining the differences in total orange contained growth temperature as well as controlling for male age and size. The next best model was within one AICc point and contained growth as well as mother's embryonic temperature (plus age and size, **Figure 5**). Total black was not described at all by my explanatory variables. There was no difference in the number of spots (chi-square (black) = 5.36, df = 9, p-value = 0.8, chi-square (orange) = 6.6, df = 12, p-value = 0.88).

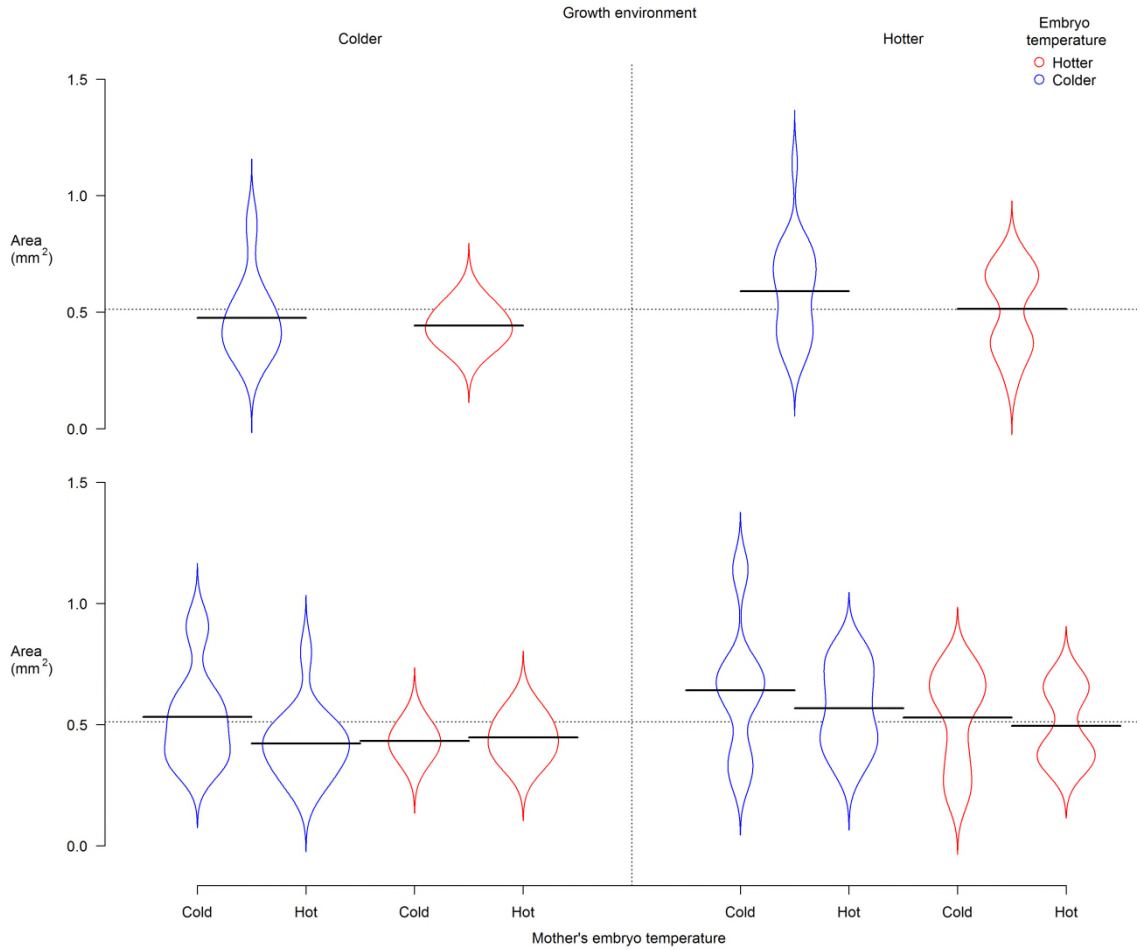

Figure 5: Total area of orange. Hotter environmental conditions led to an increase in total colouration (top). There was also an indication that mothers whose embryonic temperature was colder produced males with a larger area of orange too (bottom).

The number of orange or black spots was not significantly different between fish however the mean size of spot did differ as a result of temperature **(Table 2, Figure 6)**. With male age and size as covariates, model selection led to a model (adjusted $R^2 = 0.151$, $F(5, 70) = 3.657$, p-value < 0.01) including growth (hotter) ($\beta = 0.0032$, $t = 1.768$, p-value < 0.1), embryonic (hotter) ($\beta = -0.0041$, $t = -1.97$, p-value < 0.1) and mother's embryonic temperature (hotter) ($\beta = -0.006$, $t = -3.477$, p-value < 0.001) to describe the size of orange spots.

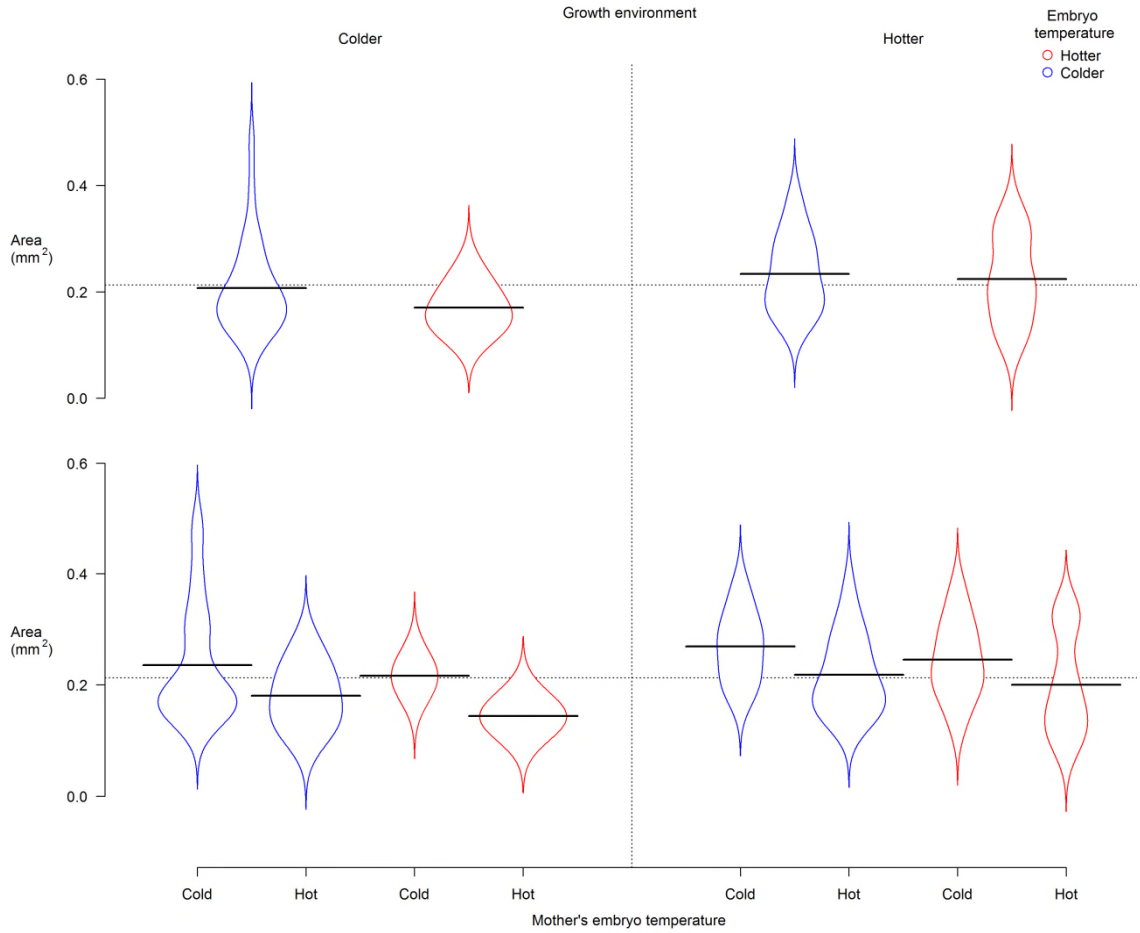

Figure 6: Mean size of orange spot across all 8 treatments. Hotter environmental and colder embryonic conditions (both their own and their mother's) are linked to larger orange spot size.

Embryonic temperature was important in explaining the variation in black spot size with hotter conditions as an embryo leading to larger black spots (β = 0.0014, t = 2.287, p-value < 0.5, **Figure 7**). This model was the best one using my covariates however it did not explain very much of the observed variation in the data (adjusted R^2 = 0.034, F (3, 70) = 1.86, p-value =0.144).

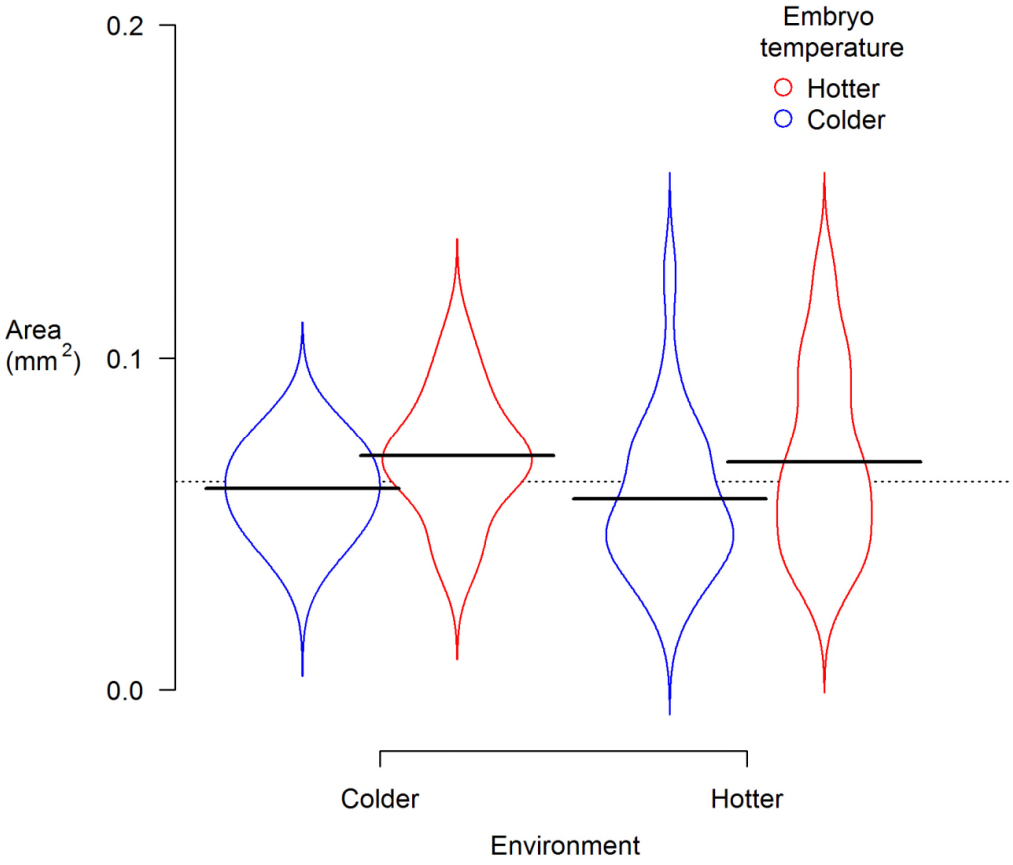

Figure 7: Differences in mean black spot size between fish in each treatment. Hotter embryonic conditions led to greater black colouration.

Results – Behaviour

Table 3: Number of replicates per treatment in generation 1 and 2 with average number (count) of *Displays* and *Sneak* attempts, plus standard deviation (sd). The letter couplet indicates the *embryo* and *growth* temperature experienced in each treatment.

	Generation 1				**Generation 2**			
	CC	**HC**	**CH**	**HH**	**CC**	**HC**	**CH**	**HH**
Behaviour replicates	14	12	13	7	29	12	23	15
Display	11.6	6.9	8.1	8.3	7.9	5.8	6.2	5.7
sd	6.3	4.3	5.5	4.2	6.5	3.4	4.8	3.4
Sneak	4	3	3.8	2.9	4.1	2.9	3.6	2.3
sd	2.4	2.3	1.3	1.9	2.6	3.0	3.0	1.5

Display behaviour

In both generation one and two, variation in display behaviour within a treatment was high and subsequently there was no combination of covariates that outperformed the null model in explaining display behaviour **(Table 3)**.

Sneak behaviour

Sneak behaviour in the first generation was best described by a model including embryonic temperature only, with hotter temperatures leading to fewer sneak attempts ($\beta = -0.287$, $z = -1.735$, p-value < 0.1, **Figure 8**). This model, however, explained a very small amount of variance (adjusted $D^2 = 0.0328$) and there were multiple alternative models with similar levels of support.

In generation 2 there was no discernable effect of mother's embryonic temperature and once removed there was a similar pattern to sneak behaviour seen in generation one **(Table 3, Figure 8)**. The model including embryonic

temperature was again the best model describing sneak behaviour (β = -0.399, z = -2.878, p-value < 0.01) with sneak behaviour reduced in fish from hotter embryo treatments. Once again, however, the variance explain was very small (adjusted D^2 = 0.0389) and there were multiple models within a few QAICc points suggesting the picture is not too clear.

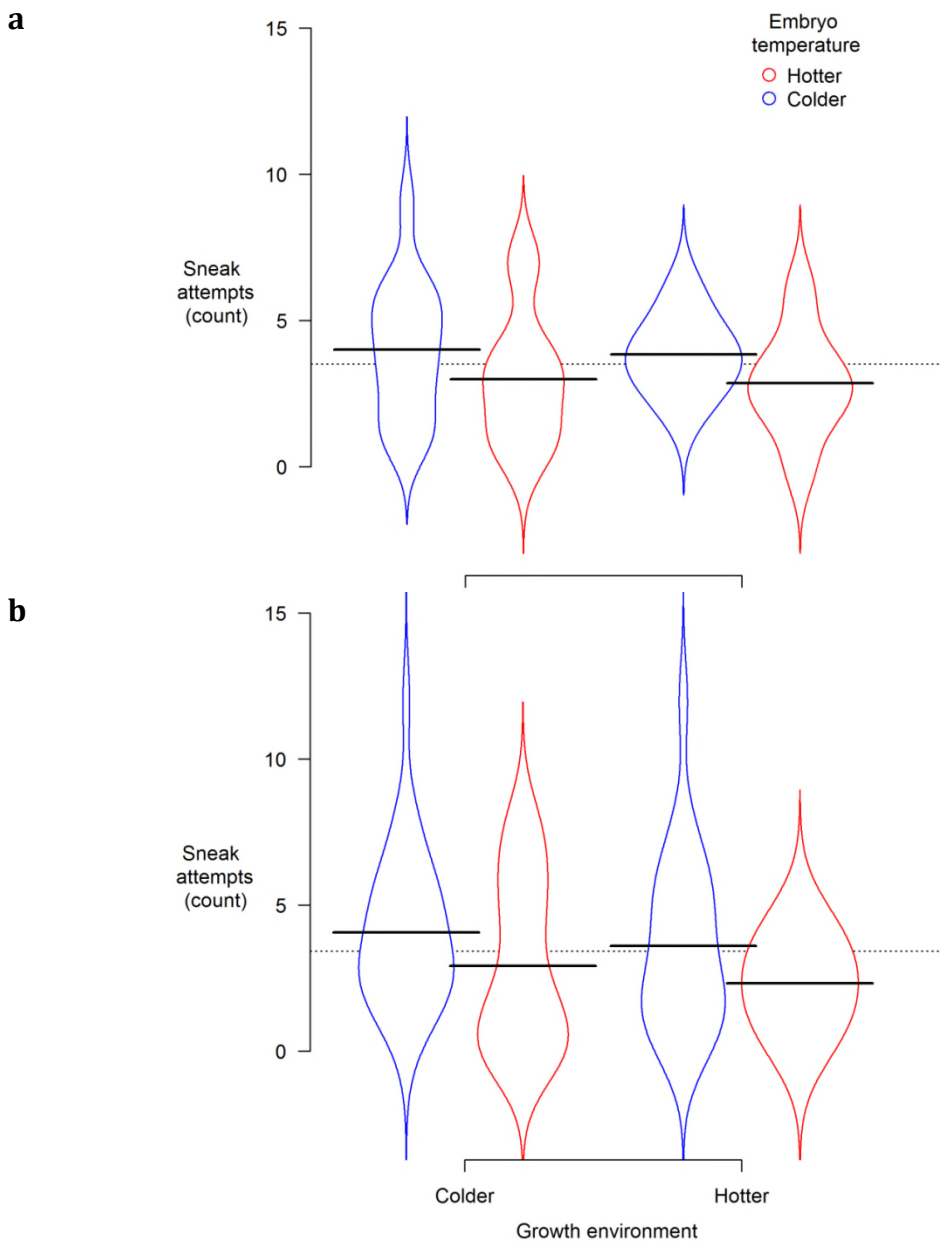

Figure 8: Sneak mating attempts in **a)** generation 1 and **b)** generation 2. Colder embryonic conditions led to higher sneak mating attempts.

Results – Sperm

Table 4: Sperm size characteristics (plus standard deviation) for each treatment from the fish in generation 1 and 2. The letter couplet indicates the *embryo* and *growth* temperature experienced in each treatment.

	Generation 1				**Generation 2**			
	CC	**HC**	**CH**	**HH**	**CC**	**HC**	**CH**	**HH**
Sperm size replicates	8	11	6	7	24	9	19	14
Head (µm)	3.25	3.26	3.22	3.12	3.07	3.05	3.02	3.04
sd	0.12	0.18	0.22	0.25	0.21	0.21	0.27	0.24
Midpiece + Tail (µm)	37.47	38.09	36.77	35.69	35.16	35.28	34.15	35.25
Sd	1.48	1.46	2.33	1.84	2.67	1.85	2.32	2.62

Sperm head size was not well described by my covariates with no model outperforming the null model. Hotter growth conditions led to shorter sperm tails, however **(Table 4, Figure 9)**. The best model (adjusted $R^2 = 0.231$, $F(2,29) = 5.667$, p-value < 0.01) explaining the observed differences in tail length included environment temperature (hotter), $\beta = -1.516$, $t = -2.53$, p-value < 0.05 as well as male age $\beta = -0.044$, $t = -1.94$, p-value < 0.1.

For the sperms measured from fish in generation 2 there was no model that was better than the null model in explaining any of the characteristics measured **(Figure 9)**.

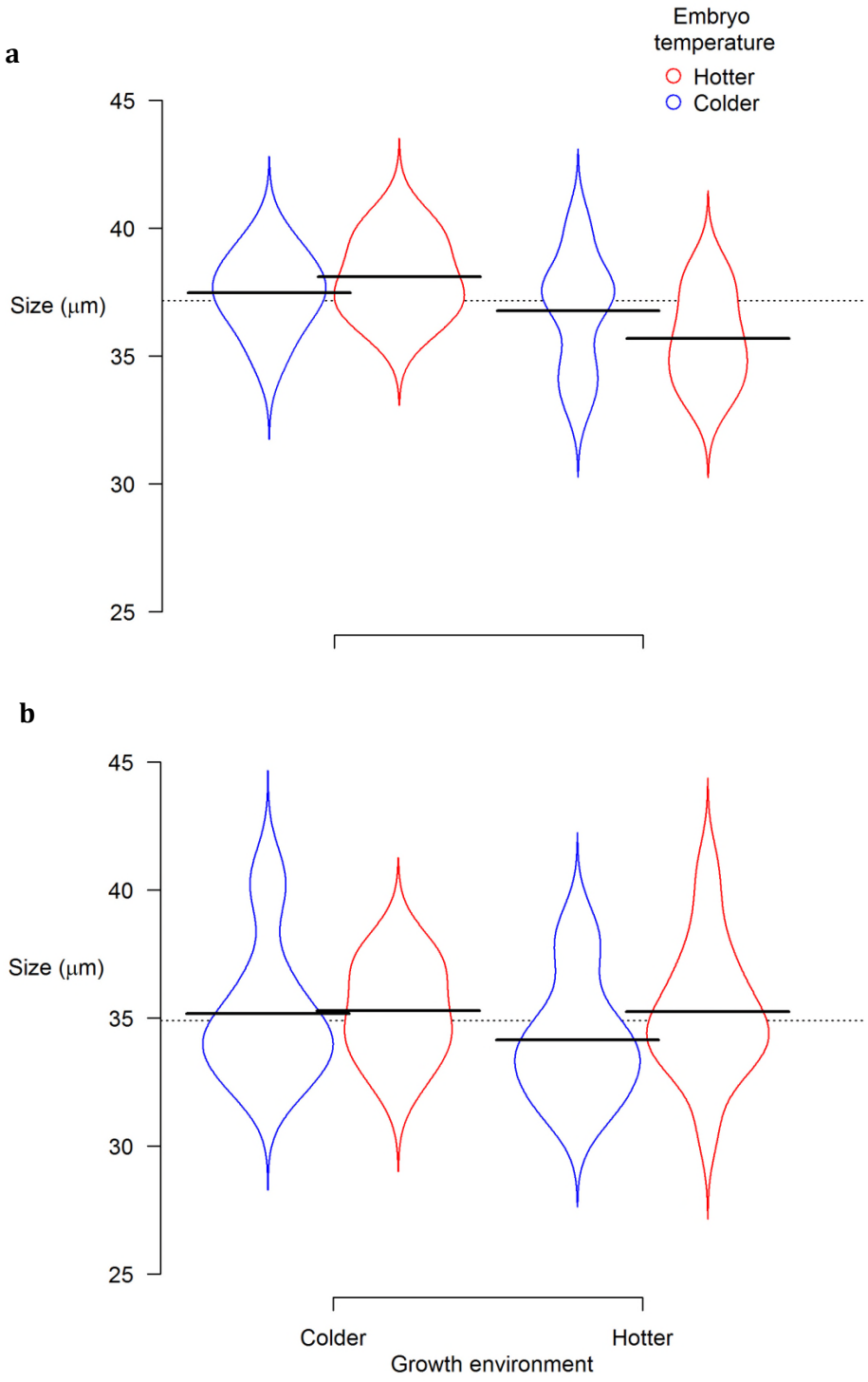

Figure 9: Sperm tail length in **a)** generation 1 and **b)** generation 2. Sperm tails were significantly shorter in the hotter growth environment in the first generation but not different in generation 2.

Discussion

This series of investigations aimed to identify changes in sexually selected traits in male guppies over successive generations. Mating behaviour once again proved variable and resistant to changing environmental conditions while sperm length became shorter in hotter conditions then similar across treatments by generation two. Orange and black colour, however, was affected by thermal regime and temperature had regular although inconsistent effects.

As with the previous chapters, mating behaviours appeared quite resilient to thermal change. Display behaviour was variable between individuals but broadly consistent between treatments and across generations. Sneak mating seemed to be affected by embryonic temperature however, like with the display behaviour individual variability was great enough to mask any statistically significant differences. Increased replicates may have provided a clearer answer however data from previous chapters suggests that mating behaviours will continue to be performed across a wide range of temperatures. This is positive from a species survival in the wild point of view but is not terribly illuminating when attempting to use mating behaviour as a whole-body fitness metric. A measure of mating success either through fecundity or high speed video analysis to count successful sperm transfers would enable conclusions on the fitness consequences to be more robust.

Sperm characteristics were broadly similar across treatments with solely tail length expressing significant differences between treatments. In generation one the hotter environment resulted in shorter sperm tails although this difference

was no longer present by the second generation. The sperm in this experiment were slightly smaller than sperms measured in other experiments **(Table 5)** although with similar variability suggesting this is just a population difference.

Table 5: Sperm length measurements in this, and other, studies.

Study	**Measure**	**Length (μm)**
(Skinner and Watt 2007)	Flagellum length	45.76 ± 1.56
Pitcher et al 2007	Midpiece+Flagellum	41.2 ± 3.1
Breckels and Neff 2013, 2014	Midpiece+Flagellum	51-55
This study	Midpiece+Flagellum	34-38

It may be that sperm length is a canalised sperm trait and that two generations is too short a time scale to expect any significant change in length. In the study by Breckels and Neff (2014) it took a year (~4 generations) of thermal exposure for the sperm length of guppies to decrease by a few μms. Interestingly, though, this size did not significantly change further over the following six months again suggesting it is not directly affected by temperature.

The midpiece of the sperm is packed with mitochondria which provide the adenosine triphosphate (ATP) necessary to power sperm motility. One might expect the number of mitochondria to be higher and therefore the midpiece longer in colder temperatures to counter the reduction in metabolic rate (Guderley and St-Pierre 2002). Conversely the metabolic activity may result in increased oxidation by free radicals and thus have a negative effect on size or, more likely, motility. It was impossible using light microscopy, however, to accurately distinguish the midpiece from flagellum and so the effect of temperature on

midpiece size could not be fully investigated. It is likely though that motility, rather than size, may provide a better approximation of the effect of temperature as the mechanism involved in powering sperm is so readily affected by temperature.

The effect of temperature on orange and black colouration was varied. In the first generation, total colour and total orange did not differ however there was less black in hotter environments. The number of orange and black spots did not differ but in hotter growth conditions mean orange spot size was larger and mean black spot size was smaller. In the second generation, the total colour of fish was highest in hotter environments and this was due to a significant increase in total orange. Total orange and mean orange spot size was larger in hotter growth conditions and, intriguingly, colder maternal embryonic conditions. This requires further investigation to understand the veracity of the relationship **(Table 6)**.

Table 6: Significant effects of 'hotter' temperature exposure, at different ontogenetic stages, on male colour characteristics (effect of 'colder' is the reverse). Embryo = embryonic temperature (same as mother's growth temperature), Growth = growth temperature, Mother's embryo = embryonic temperature experienced by a male's mother.

	Generation 1		**Generation 2**		
	Embryo	Growth	Mother's embryo	Embryo	Growth
Total colour					↑
Total orange					↑
Total black		↓			
# spots					
Mean orange spot size		↑	↓	↓	↑
Mean black spot size		↓		↑	

Colour patterns and area of orange have strong heritable components in guppies (Houde 1992) which could explain why there were no differences in the

number of colour spots. Despite this, thermal experience had significant effects on their relative sizes demonstrating a phenotypic component to colour pattern. As sibling groups were split between treatments the effect of heritability will have been removed from the results.

Melanin pigments have varied roles in photoprotection and thermoregulation, and as antioxidants and immunostimulants (reviewed in McGraw 2005). Melanin production may be limited by oxidative stress (Schallreuter et al. 2008) and so the pattern observed in generation one could be a response to the oxidative stress resulting from higher temperatures, although no such effect was maintained in the successive generation. Orange colouration in guppies is produced by a combination of carotenoids and pterin pigments (Grether et al. 2001a) and while carotenoids have been linked with an antioxidant role (Krinsky 2001, Stahl and Sies 2003) a similar role for pterins remains unclear (McGraw 2005). Food, and therefore dietary carotenoids, was not limiting in this experiment and so it is possible that increasing areas of orange in hotter temperatures may signify enhanced antioxidant defences (reviewed in Perez-Rodriguez 2009 although uncertainty in this field remains) however this would need further investigation to determine. Unlike carotenoids, pterins can be synthesised and so alternative colour variables such as hue or saturation which are augmented by pterins are therefore condition dependent (Grether et al. 2001a) and consequently may be more informative about the effect of temperature.

Collectively, increased temperature led to an increase in orange and a decrease in sperm length in generation one but no discernible effect in mating

behaviours nor in any measured trait in generation two. Temperature has pervasive effects over all aspects of ectotherm life history and to record very little response in these traits suggests that 1) they are canalised and inflexible to change over such a short time scale or 2) the thermal regimes were not extreme enough to cause any differences outside of the guppy's natural phenotypic flexibility.

Increasing temperature does not necessarily have to have an immediate negative effect. The costs associated with maintaining size and growth characteristics across thermal regimes (if there are any) may only be apparent in longer term effects. Oxidative stress resulting from a higher metabolic rate could lead to a reduction in total lifetime reproduction or lifespan as a consequence of an increased allocation of energy to antioxidant defences (Metcalfe and Alonso-Alvarez 2010, Hemmer-Brepson et al. 2014). Similarly, many ectotherms are predicted to live at temperatures slightly below their optimal fitness to allow for flexibility in response to temperature change (e.g. Martin and Huey 2008) and there are studies that have shown ectotherms can benefit from long term climate increases (Huang and Pike 2011, Clarke and Zani 2012).

It is possible, therefore that fluctuating temperatures in the guppy's natural environment have established a thermal tolerance and phenotypic flexibility which enables them to withstand high temperatures with little cost in fitness. So, with guppies a highly successful invasive species established in many countries around the world, the question becomes, what is the lower thermal limit restricting poleward range expansion in guppies and what are the fitness consequences of living in colder thermal environments?

Discussion

Discussion

Responding to novel environmental conditions, for example when facing a changing climate or invading new habitats, is an important challenge. Increasing global temperatures and occurrence of extreme weather events are two sources of climatic pressure operating at different timescales which species will need to overcome in order to ensure their continued existence over the coming century.

Understanding what the effects are and how species react to a change in environmental conditions is essential to predicting the outcome of these events and is an integral component for minimising or managing negative consequences.

The aims and main results of this thesis were:

- Describe how natural variability in abiotic conditions affects daily patterns of mating activity in wild fish **(Chapter Two).**

This experiment investigated the effects of naturally occurring abiotic variability on daily patterns of male mating activity. The results demonstrated that water temperatures in Trinidad can vary up to 10°C in a 24 hour period and ranges between 23 and 34°C**.** Light and temperature levels influence the frequency of mating activity differently according to the time of day yet male guppies continue to perform mating behaviours across all light and temperature levels.

- Establish what the immediate effects of temperature change are on mating behaviours **(Chapter Three).**

In this investigation I attempted to determine the breadth of temperatures over which male guppies perform mating behaviours. The expectation was that this range would be similar to the temperatures guppies experience in Trinidad however it was, in fact, much broader. Males maintained their interest in females over the entire test range of temperatures (from 17-34°C) and continued to attempt to mate in temperatures much lower than they experience in the wild.

- Investigate how thermal acclimation alters performance in mating behaviour, growth and offspring traits at different temperatures **(Chapter Four).**

Acclimation is a phenotypic response to changing thermal conditions which takes place in order to minimise any loss of performance in the novel conditions. Guppies successfully acclimated to the 24°C and 27°C treatments and accordingly the peak of mating behaviour was greatest at the temperature in which they were acclimated. Conversely, the 30°C treatment – a temperature routinely experienced in Trinidad – caused high adult mortality and offspring production to cease. Higher temperatures (27°C and 30°C) led to increasingly smaller offspring as exposure continued. The temperature experienced during development as well as during juvenile stages affected the speed of growth and final adult size with lower *developmental* and higher *growth* temperatures leading to larger fish and faster growth. These results demonstrate the effect of temperature on life history traits and provide an indication of the guppy's phenotypic flexibility enabling it to withstand thermal change.

- Explore what the effects of environmentally-realistic fluctuating temperatures are:
    - on life history traits **(Chapter Five)**
    - and on sexually selected traits **(Chapter Six)**.

Environmental temperature naturally fluctuates over a daily cycle and so I created two ecologically realistic thermal regimes in order to assess the life history consequences over successive generations of entering novel conditions. In the first generation both male and female growth were affected by thermal regime with warmer temperatures generally resulting in smaller individuals and smaller offspring. Offspring continued to be larger in colder conditions but the effect on growth was no longer significantly different in the second generation. Mating behaviour continued to be resilient to thermal change and sperm length was inconsistently affected by thermal regime. Orange colouration exhibited a more apparent connection to temperature with warmer conditions resulting in larger areas of orange.

The effect of switching thermal conditions between development and growth resulted in the measured traits being intermediate between the consistently higher and consistently lower treatments. Furthermore, despite routinely experiencing temperatures that interrupted reproduction in **Chapter 4** the fish in this experiment were able to successfully produce offspring indicating a difference in the effect of stable and fluctuating temperatures.

Outcomes

Temperature in the guppy's natural environment is varies widely over a daily cycle

There is a broad geographic pattern of temperatures increasing in variability with decreasing latitude (e.g. see Sunday et al. 2011). I found that water temperatures in Trinidad, however, routinely vary by as much as 10°C over a daily cycle, a range much greater than experienced by temperate freshwaters (e.g. Caissie et al. 2005). Conversely, seasonal change in temperate systems can be in the order of 20°C (e.g. Caissie 2006) while in Trinidad there was limited change to the daily average although daily variation was different during different times of the year. Species must adjust or adapt to this variability and a common result is a generalist life history strategy (Gabriel et al. 2005). Heterogenous environments maintain genetic variability and enables generalist species to inhabit a broader niche and have greater tolerances to abiotic factors (Kassen 2002). Being a generalist however may come with the cost of lower performance levels than a specialist when compared in the same environment (Huey and Hertz 1984).

The daily variability in temperature in Trinidad operates over a much shorter timescale than the majority of physiological changes that acclimation requires. Consequently, this may have resulted in the broad thermal tolerance which the guppy possesses. Similarly, environmental heterogeneity may have led to a preadaptation to novel environments (Ketola et al. 2013) which has contributed to the success of guppies as an invasive species.

Guppies respond to thermal change with broad phenotypic plasticity

Temperature has a fundamental effect on ectotherms and guppies were no different. Life history traits including daily activity, growth, offspring size, and colouration all responded to changing thermal conditions. We can, therefore, conclude that changing thermal conditions, or introduction into novel environments, will have life history consequences for the guppy. As these traits all influence survival and reproductive success they can subsequently be used to make predictions about fitness and ecological success. Furthermore, the effect of thermal environment on life history traits implies that guppy populations, which experience different thermal conditions, will exhibit different life histories (see **'The guppy system'** section below).

The phenotypic flexibility and rapid adaptability of guppies has been well described in response to predators, social environment, light and resource availability among others (see Meffe and Snelson 1989, Magurran 2005, Evans 2011) The results within this thesis provide further evidence of plasticity, in this case thermally induced. Exposure to different temperatures resulted in phenotypic changes which altered growth, offspring size and number, male colour and daily activity patterns. While ontogenetic thermal exposure is capable of causing permanent phenotypic changes (e.g. Wilson and Franklin 2002, Beldade et al. 2011), in these experiments *environmental*/*growth* temperature had the strongest effect.

The consequence of the guppy's broad phenotypic plasticity is that they are able to withstand changes to their thermal environment. Guppies in this thesis were capable of tolerating exposure to a 17°C range of temperatures **(Chapter 3)**, were capable of acclimating to a novel temperature **(Chapter 4)** and were also able to persist in and produce viable offspring when entered into the thermal regimes spanning 13°C in **Chapters 5** and **6**. The guppy's responses to thermal change in these experiments took place over a relatively short time frame with the impact of thermal exposure apparent within the guppy's lifetime. As adaptation to novel conditions takes multiple generations, phenotypic flexibility and a generalist life history will increase the likelihood of survival in new environments.

Difference between stable and fluctuating temperatures

When guppies were exposed to stable 30°C temperatures **(Chapter 4)** the temperature was apparently too extreme and led to significantly higher adult mortality compared to the other treatments and an eventual cessation in offspring production. When routinely exposed to >30°C for a short period each day, as guppies are in the wild **(Chapter 1, Appendix 1)** and as in the experimental conditions reported in **Chapters 5** and **6,** reproduction is not inhibited and mortality is insignificant. There is evidently an effect of the duration and range of temperatures experienced by guppies which causes the differences in mortality and reproductive failure between the stable and cyclical temperatures.

There is mixed evidence for whether fluctuating temperatures have a more positive or negative effect on biology than stable temperatures. Growth in sockeye salmon, for example, has been demonstrated to be higher in cyclical temperatures

than stable high or low temperatures with the differences suggested to be because respiration rate is higher at stable high temperatures and defecation and excretion higher at lower temperatures (Biette and Geen 1980b, a). The conclusion being that, given appropriate food rations, fish gain a bioenergetics advantage when exposed to temperatures that vary over a consistent pattern. Fluctuating temperatures also provide periods of benign temperature exposure which allows an organism to recover or repair damage caused by stressful temperatures (e.g. Lalouette et al. 2011, Chen and Stillman 2012). There is, however, contradictory evidence which indicates that daily fluctuating temperatures causes poorer energetic conversion efficiency than in stable temperatures. The consequences include higher oxygen consumption rates (Lyytikainen and Jobling 1998), increased food consumption (Spigarelli et al. 1982) and a reduction of age and length at maturity (Dhillon and Fox 2007). As guppies evolved in cyclically fluctuating temperatures it can be hypothesised that they are adapted to thermal variability and are more capable of withstanding the effects of fluctuating over stable temperatures.

Ultimately though, this debate is slightly trivial. Temperatures fluctuate in natural environments and so investigations employing fluctuating temperatures that resemble natural conditions, will produce more realistic conclusions. The ecological realism of model parameters obtained from experiments is important for improving the accuracy of the predictions attempting to model the biological consequences of climate change for species (for examples see also Folguera et al. 2009, Niehaus et al. 2012, Beauregard et al. 2013). Therefore, realistic experimental conditions should be encouraged.

Males attempt to mate at physiologically damaging temperatures

Temperature had immediate and long lasting effects on a number of the traits I measured, yet the motivation of males to attempt courtship with females continued at all test temperatures. The optimal temperature for each behaviour was slightly different **(Chapter 3)** however the variability around the data was such that the 80% performance range spanned ~7°C and reproductive motivation was recorded at all temperatures. A similarly broad range was observed in **Chapters 2**, **3** and **4**.

While the negative effects of lowered temperatures may be negligible (until they drop below the point where cold shock becomes an issue), the effect of elevated temperature have been demonstrated for guppies. Muñoz et al. (2012) determined that at 30°C guppies pass a physiological '*tipping point*' where acclimation was no longer capable of negating physiological stress. I observed a similar response in **Chapter 4** when 30°C resulted in elevated mortality and reproductive failure. Despite the physiological stress guppies are under from elevated – or lowered – environmental conditions, male fish continued to attempt to inseminate females. It could be speculated that the Darwinian fitness benefits of mating outweighs the energetic cost when temperatures are not optimal. Sneak mating and courtship displays differ in effectiveness and energetic costliness both of which will be further modulated by environmental temperature. Whether mating behaviour equates to fertilisation success at all temperatures and how quickly and effectively sperm stores are replenished, however, remains unclear.

Thermal tolerance is broader than ancestral history would suggest

One of the more intriguing results from this work – and supported by records of successful guppy invasions around the world – is that guppies are capable of tolerating temperatures lower than they commonly experience in Trinidad. Guppies in Maravatio, central México, for example, are present in water that averages 19°C while the lowest temperature I recorded in Trinidad was around 23°C **(Appendix 1)**. The poeciliid fish from which guppies later diverged originated in Northern and Central South America around 44 million years ago (Hrbek et al. 2007). Guppies therefore evolved in a tropical environment and it appears unlikely that they have a recent ancestor which evolved in temperate latitudes to explain the guppy's cold tolerance.

Synthesis and conclusions

Environmental relevance and realism

The experiments related in this thesis provide detailed measures of how changes to the thermal environment affect aspects of the guppy's life history. Attempts were made to make the experiments ecologically realistic by using semi-natural conditions **(Chapter 2)** and thermal regimes which resemble the guppy's natural environment **(Chapter 5** and **6)**.

The thermal regime(s) used to investigate the effect of temperature is important. Many ecological studies have used average mean temperatures (see Clusella-Trullas et al. 2011) which leads to a 'functional' norm – describing the response of an organism to a specified temperature – rather than a 'realised' reaction norm generated by exposure to naturally variable conditions (Paaijmans et al. 2013). As temperature is rarely stable in natural systems this can result in erroneous conclusions based on under- or over-estimates of the effect of temperature on important life history variables (e.g. Bozinovic et al. 2011, Carrington et al. 2013). Consequently, as environmental variation can affect many aspects of life history beyond the effect of mean temperature alone, ecologically relevant temperatures should be encouraged in studies investigating the effect of temperature or those hoping to make realistic predictions for the future (Folguera et al. 2011, Niehaus et al. 2012).

Furthermore, to improve the power of predictive models it is important to investigate relevant traits that are either essential for surviving change or are particularly susceptible to changing conditions. Reproductive traits have been

labelled the 'Achilles' heel' of tropical ectotherms (Zeh et al. 2012) as egg/offspring size (Atkinson 1994), clutch size/fecundity (Smith and Fretwell 1974), sex steroid production (Miranda et al. 2013), oogenesis and spermatogenesis (Dorts et al. 2012) are all particularly susceptible to temperature. Moreover as traits are not independent from each other, incorporating multiple traits will provide more realistic information on phenotypic and adaptive responses (Foray et al. 2014).

In my experiments I chose to record traits related to reproductive success and life history each of which can easily be linked to fitness effects. Individually housing fish in **Chapter 5** and **6** enabled me to generate individual growth trajectories which could be accurately related to age. This approach would also lend itself to investigating the heritability of traits through quantitative genetic analysis. Moreover, the majority of measures were made using a whole-animal approach which can be more directly linked with ecological success (*cf.* Huey and Stevenson 1979). Ecological and physiological trait-based approaches to predicting the effects of climate change and invasive spread has undergone a recent increase in attention (e.g. Janion et al. 2010, Angert et al. 2011, Diamond et al. 2012, Bernardo 2014). Whole-body, as opposed to cellular-level, approaches can be more easily used to compare responses observed in both the lab and field. Furthermore, it is possible to infer the effect on functional role and consequently compare fitness related effects between species.

The guppy system

The guppy has long been used as a model species investigating evolutionary ecology and, in particular, the effect of predation on life history traits. The geography of the guppy's habitat in the North of Trinidad is such that the rivers which guppies inhabit are punctuated by waterfalls which act as natural barriers allowing for only downstream migration. The further upstream one progresses the fewer guppy predators are present and a great body of work has demonstrated the effect of this predation gradient on guppies (e.g. Liley and Seghers 1975, Reznick and Endler 1982, Shaw et al. 1991, Reznick et al. 2001). Guppies in upstream populations tend to grow to larger sizes and male guppies are more colourful, while downstream populations consist of smaller fish and males that perform more sneak mating than colourful displays (see Houde 1997).

The water in upstream, "low predation" habitats is also commonly cooler, faster flowing, more shaded and less productive than the downstream, "high predation" equivalents (Kenny 1995, Magurran and Phillip 2001). Furthermore, along the waterway there is variation in habitat quality, differences in population size and organismal diversity (Liley and Seghers 1975). Accordingly there are studies that have indicated that environmental properties of habitat also influence the biological and life history characteristics of guppies (Endler 1995, Grether et al. 2001b, Reznick et al. 2001, McKellar et al. 2009, Torres-Dowdall et al. 2012b).

The thermal regimes I created in **Chapters 5** and **6** resemble those found just 10 kilometres apart in Trinidad **(Appendix 1).** Although the average temperature in my treatments differed by just 3°C, the cyclical nature of it led to

life history changes comparable to what is observed in the wild, and without predators (e.g. in warmer conditions fish matured at a smaller size, females produced more offspring for their size, and of a smaller size). The results from this thesis indicate that predation may not be the singular reason for the evolution of wild guppy life history traits and that gradient and variability of water temperature in Trinidad warrants further investigation.

Furthermore, it is apparent that stable and fluctuating temperatures have differing effects on traits although it is unclear whether the differences observed are due to the temperature range, thermal extremes or the duration of exposure to each temperature. The fluctuating temperatures used in these experiments were similar to naturally occurring conditions in Trinidad and so resemble the conditions to which guppies are adapted. Similarly, guppies may take advantage of the regular periods of benign temperatures to recover from extreme or detrimental temperatures and thus ameliorate stress. Additionally, periodic exposure to elevated temperatures may actually improve performance. If the optimal body temperature of guppies is positioned at a slightly lower temperature than the fitness maximum (*cf.* 'sub-optimal is optimal' Martin and Huey 2008, see also **Introduction**) the increase in temperature would result in a performance increase. It is further possible then that, if the temperature increase results in the fitness maximum, there will potentially be no detrimental effects of oxidative stress. It would be necessary to compare whether the fitness maximum is the same over short and whole-life timescales to really conclude if the higher temperatures were having detrimental effects or otherwise.

Ultimately, it is essential to be aware that stable and fluctuating temperatures may cause a difference in effect. This is important when comparing species responses between the lab and field or when predicting the effect of thermal change on wild species using lab-generated data. Within scientific literature there is discussion surrounding the applicability of data generated using stable temperatures in predictive modelling (e.g. Folguera et al. 2009, Folguera et al. 2011, Niehaus et al. 2012, Arrighi et al. 2013, Ketola et al. 2013, Paaijmans et al. 2013). The general consensus is that fluctuating temperatures are more realistic than stable although there is evidence that the conclusions obtained from stable and fluctuating temperatures can be similar (Fischer et al. 2011b). I would suggest that ecologically realistic thermal regimes increase the realism of results and that (for guppies at least) fluctuating temperatures lead to more naturalistic results than stable temperatures.

Because the temperature variability in Trinidad follows a regular, predictable pattern guppies may have become specialised to variable environmental conditions. This specialism could therefore have resulted in mechanisms which allow them to inhabit a range of temperatures including those below what they experience in Trinidad. Indeed, the temperatures they face in areas that they have invaded are certainly cooler than in Trinidad but may not be so low as to be physiologically damaging. The outcome of this is that temperature alone may not be limiting range expansion for guppies. Competitive interactions with endemic species better suited to the environment may be a key factor excluding guppies from expanding their range further. It would also be interesting

to investigate whether guppies are actually specialists in variable conditions or if their tolerance comes with a cost.

It is widely accepted that variability in environmental conditions results in a generalist life history strategy (e.g. Kassen 2002) and the variable habitat conditions along river systems in Trinidad have led to local adaptation by guppies (Endler 1995, Torres-Dowdall et al. 2012a, Torres-Dowdall et al. 2012b). Accordingly it can be hypothesised that the variability in thermal conditions in Trinidad has contributed to the guppy developing a broad thermal tolerance and becoming phenotypically flexible. Furthermore, this tolerance and ability to rapidly adjust to new conditions is, in part, responsible for the guppy's success as an invasive species

Guppy troubles?

Ectotherms are particularly at risk from increasing temperature as biochemical reaction rates increase exponentially with increasing temperature (Gillooly et al. 2001, Brown et al. 2004). Tropical ectotherms will therefore suffer greater absolute changes in metabolic rate per temperature increase than temperate species (Dillon et al. 2010). The asymmetric shape of thermal reaction norms means that, without migration or adaptation, a small increase in temperature could result in performance traits becoming compromised with detrimental consequences (Deutsch et al. 2008). Species living in the tropics are particularly at risk as they are already likely to be living close to their optimal temperature and consequently have a narrow *thermal safety margin* (Bozinovic et al. 2011, Paaijmans et al. 2013, Sunday et al. 2014). As the tropics are host to the greatest

ectotherm diversity, the implications of increasing temperature could be stark (Zeh et al. 2012).

The results from this thesis support previous conclusions about the phenotypic flexibility of guppies and augment them by demonstrating how guppies respond to changing environmental temperature. I have shown that guppies are capable of entering novel conditions and producing viable offspring. This means that guppies have effective mechanisms with which to tolerate broad thermal change and, by continuing to reproduce successfully, provides the opportunity for adaptation (e.g. through methylation or incorporation of mutations) to take place.

But how might this relate to the guppy's future? Predicting how species will respond and redistribute in response to climate changes is one of the most important problems to address in forthcoming years (e.g. Parmesan and Yohe 2003, Thomas 2010). The Intergovernmental Panel on Climate Change (IPCC) prediction that global temperatures will increase by up to 4°C by the end of the century (IPCC 2014) has multiple consequences for the guppy. Within Trinidad, guppy migration is restricted by barrier waterfalls and so it would not be possible for lowland guppy populations to move upstream to colder conditions if temperatures became too great. As Trinidad is an island it is also impossible for guppies to emigrate without human assistance. The result is that, despite guppies widely expanding their global range, the original populations in Trinidad – and Tobago – could be seriously impacted by a large increase in temperature.

Outside of Trinidad, an increase in temperature would increase the likelihood of guppies expanding their range and lead to more competitive

interactions with native fish assemblages. Further range expansion would increasingly place guppies in seasonal environments very different to the conditions they experience in Trinidad yet whether guppies would be able to exist in these locations depends on the thermal regimes specific to each environment. Guppies in Trinidad reproduce year-round although a move towards seasonal reproductive cycles might be one solution to surviving in seasonal environments. Colder environmental conditions cause biochemical reaction rates to slow which results in, among other effects, slower energy conversion and development times (Gillooly et al. 2002). By concentrating reproductive effort in the warmer part of the year guppies could spend the colder months focussed on foraging or systems maintenance. A gradient in temperature elicits a similar response in *Gambusia holbrookii*, a close relative of the guppy, across its invaded range between southern France and southern Spain. Higher latitude populations allocated greater energy to reproduction than lower latitude populations (Benejam et al. 2009). Additionally, seasonal temperatures determined the population size of *Gambusia affinis* with warmer seasons enabling more reproductive events (Matthews and Marsh-Matthews 2011).

The consequence of climate change for guppies both within and without Trinidad is made more uncertain by the unpredictable frequency and scale of extreme weather events. Thermal fluctuation is a routine occurrence in Trinidad and it is likely that this has resulted in guppies developing adaptations to predictably variable thermal regimes. Consequently, unpredictable events will likely represent a more significant problem as guppies have not been regularly exposed to stochastic variability. Moreover, unpredictable variability can have

ecosystem-wide effects by impacting individual fitness related effects such as predation defences and growth rates (Barbosa et al. 2014), migratory ability (Svenning and Gullestad 2002) and oxidative stress (Reyes et al. 2003). Thus the detrimental effects can be experienced by individuals themselves or as a result of disruption to the ecosystem at a different level. Furthermore, as daily variability is a more important driver of life history than mean temperature, the impact on daily thermal regime by climate change will also be a significant factor influencing the survival, or otherwise, for guppies.

During early stages of environmental change, phenotypic responses in the first few generations will determine a species' success or failure. A species' ability to behaviourally adjust to novel conditions is particularly important as it enables an immediate response which takes place more quickly than adaptation (Kearney et al. 2009, Tuomainen and Candolin 2011, Sih 2013). Guppies are certainly capable of altering their behaviour to exploit more favourable conditions. To avoid predation guppies can become active at night to forage (Fraser et al. 2004) and are willing to associate with conspecifics when placed in novel conditions (Camacho-Cervantes et al. 2014). I also showed in **Chapter 2** and Reeve et al. (2014) that males increase time spent courting females when light and temperature levels are more agreeable. This behavioural thermoregulation, whereby guppies change their activity patterns to when environmental temperature is more favourable, is one method by which guppies could adjust to changing conditions. Predicting the likelihood of success for guppies therefore depends on the specific thermal regime changes that they experience.

Conclusion

Using ecologically realistic settings I have delivered an insight into the fundamental thermal niche of a model fish species. I have also demonstrated the consequences of entering a novel thermal environment at different time scales.

This thesis has also provided detailed information on the phenotypic responses the guppy makes to changing thermal conditions. The results suggest that guppies possess a range of mechanisms that will enable them to withstand many climate change scenarios and remain reproductively active although the effect of increasing unpredictable events may be more difficult to overcome.

Climate warming will also facilitate range expansion and present the opportunity for guppies to expand into colder and seasonal environments. I have demonstrated that guppies can quickly make life history changes in response to novel environmental conditions which increases the possibility of them establishing in seasonal or temperate environments. If guppies persist in the invaded environment there are likely to be negative consequences as guppies compete with native species. Environmental managers should be aware of the guppy's invasibility and respond quickly to invasion events. The information in this thesis would be a beneficial addition to the development of environmental models predicting the biological consequences of climate change

Future directions

Lifetime fitness consequences

Changing the timescale at which fitness traits are measured will provide more information on the effects of environmental change. I have demonstrated that in the short term guppies are broadly capable of entering novel conditions, reproducing successfully and thus providing another generation. Whether there are effects that operate over a lifetime scale (such as lifetime fecundity or number of offspring sired) will determine if there are individual-level fitness consequences. These consequences will determine the success, or otherwise, of establishing a new population in new environmental conditions.

Different measures of fitness

If a similar investigation were to be repeated but lifetime fitness measures were unfeasible there are alternative fitness traits that could be measured to provide insight into the immediate effects of changing temperatures.

- A condition index, rather than standard length alone, may have indicated further differences between treatments. Assuming fish are fed to satiation, larger muscle mass or body depth would suggest more resources spent on growth and performance benefits could include faster swimming speed, greater stamina, and increased fecundity in females.

- Similarly, a histological analysis of muscle fibres would complement the growth data and potentially provide a physiological explanation for the differences in growth rate and final size between treatments. The number

of muscle fibres is modulated during development while their size (diameter) is altered by environmental conditions and feeding regime post birth/hatching (Johnston 2006). Using the same experimental design, it would be possible to distinguish the effects of developmental and growth temperature on muscle fibres and may provide an explanation for the differences in growth observed between fish in generation 1 and 2.

- The effect of temperature on guppy sperm morphology has been mixed (**Chapter 6**, Breckels and Neff 2013) likely because there is a genetic component to sperm length. Furthermore, there are counterintuitive indications as to the effect sperm length has on fertilisation success as longer tails has been linked with slower sperm (Gasparini et al. 2010) while elevated temperatures has resulted in shorter and – by inference – faster sperm (Breckels and Neff 2013). Sperm motility is a trait that is more easily linked to fertilisation success, and consequently fitness, particularly in a species with high levels of female multiple mating and sperm competition. Mitochondria within the midpiece of sperm synthesise adenosine triphosphate (ATP) through oxidative phosphorylation which is used to power sperm movement. ATP production is consequently sensitive to temperature and be affected over a much shorter time scale than morphology. Higher temperature will bring about faster ATP generation and potentially greater motility although as mitochondria also produce reactive oxygen species (ROS), increasing temperature may also result in increased oxidative stress. In mammals, oxidative stress outstripping ROS

scavenging can cause infertility (Agarwal and Sekhon 2011) although there is evidence that sperm may require some ROS in order to achieve fertilisation (Griveau and Le Lannou 1997), and promote capacitation and the acrosome reaction (Griveau et al. 1995). Boschetto et al. (2011) demonstrated that guppies with faster (and more) sperm fathered a greater number of offspring and so the effect of temperature on motility results in genuine fitness effects.

Nature vs nurture (heritability)

The experimental design (individually housed fish, sibling groups split between treatments) would allow for phenotypic traits to be compared between siblings and, in generation 2, between parent and offspring. It might then be possible to separate the effect of the environmental conditions from genetics in phenotypic expression.

Effect of lower temperatures

Guppies are seemingly capable of tolerating an increase in environmental temperatures as long as there are regular periods where temperature is lower than 30°C. However, further range expansion into sub-tropical and temperate areas will expose guppies to temperatures that are seasonal and, more likely, lower than those experienced in Trinidad. Lower temperatures cause slower biochemical reaction rates and consequently growth and offspring development takes longer to complete (Gillooly et al. 2002). Guppies in Trinidad reproduce year round however this may be impossible in seasonal environments where food or favourable conditions are limited. Investigating the life history effects of entering lower

temperatures will illuminate the fundamental effect of temperature and also provide life history information which will be important for identifying the potential consequences of guppies invading new environments. Other poecillids have invaded seasonal (Meffe 1990) and temperate (e.g. Benejam et al. 2009, Vidal et al. 2010) systems with a tendency towards seasonal life histories; one would expect a similar response by guppies.

Do the observed phenotypic effects become adaptive?

Given adequate time and space, the guppy lends itself to investigating whether the plasticity that enables them to withstand exposure to novel environments is adaptive and over what timescale. The experimental system described in Chapters 5 and 6 worked effectively and modern advances in molecular science have resulted in a developing knowledge of the guppy genome and transcriptome (e.g. http://guppy.weigelworld.org/weigeldatabases/, Willing et al. 2010, Fraser et al. 2011, Sharma et al. 2014). It would therefore be possible to expose populations of guppies to novel environmental regimes and follow the molecular changes that take place. This would indicate the molecular changes of entering novel environments and also the number of generations required for populations to adapt.

Field based research

It is essential to know if the daily cycles of temperatures I recorded at two sites within one river are repeated in other water courses in Trinidad (and elsewhere in the tropics). It would also be interesting to record temperatures over the full length of a river to obtain a clearer picture of longitudinal thermal change.

Furthermore, a detailed inspection which mapped thermal variation and microhabitats at individual sites would be important in determining whether there are thermal gradients within a location which would provide the opportunity for behavioural thermoregulation. Once the thermal map has been created it would then be possible to record guppy movements and determine whether they, or other species, are exploiting them.

This thesis has provided data with which to make realistic hypotheses that can be tested with wild fish populations. Confirmatory fieldwork at the geographic and thermal range edge of guppy populations will provide valuable insight into how realistic the lab based predictions are. Furthermore fieldwork in these locations will enable investigation into the effects of lower temperatures and seasonal environments on guppies.

It would also be of great interest to compare the responses of guppies with other members of the poeciliidae family and other fish that fill similar functional roles in the ecosystem. This would give an indication of the applicability of these findings across species and add weight (or otherwise) to their use in predictive modelling.

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Appendix 1

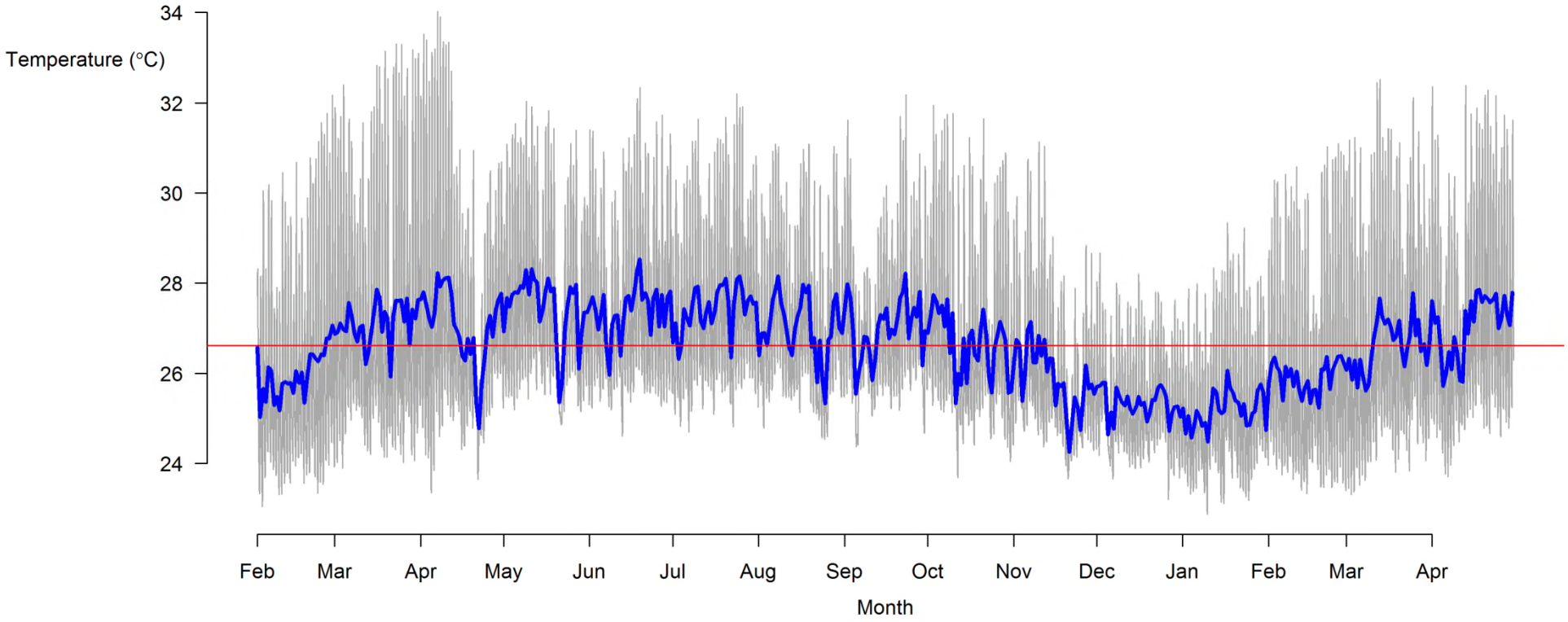

Figure 1: Daily variation in water temperature (grey line) in the Acono River in Trinidad at a site where guppies are found. Blue line is the daily mean and red line is the overall mean. Temperatures were taken every 30-60 minutes using a data logger (MicroLite lite5016, Fourier Systems).

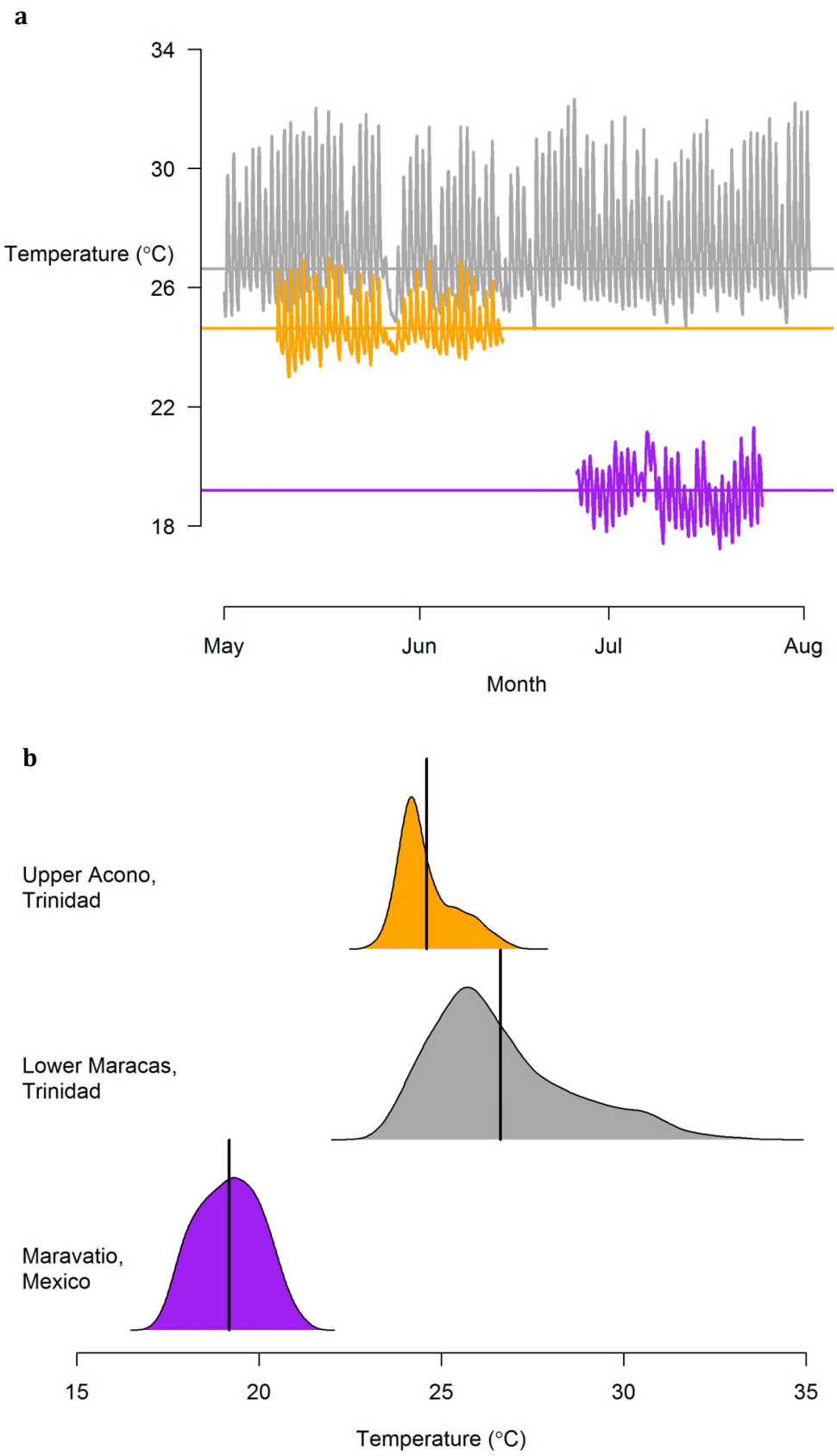

Figure 2: a) Water temperatures recorded every 30-60 minutes using dataloggers at locations in Trinidad and México where guppies are present. Horizontal line is overall mean. **b)** Density plots of water temperatures in three locations. Upper Acono (orange) and Lower Maracas (grey) are sites on the same river separated by ~10km, Maravatio is a river in the state of Michoacán, central México (purple) . Black vertical bar is the mean value.

Table 1: Temperature characteristics, at locations in Trinidad and México, where guppies are found.

	Maravatio, México	Upper Acono, Trinidad	Lower Maracas, Trinidad
Mean	19.19	24.63	26.62
Median	19.21	24.39	26.18
sd	0.87	0.80	1.96
Range	4.09	4.41	11.14
Min	17.23	22.99	22.88
Max	21.32	27.4	34.02
Count	708	1722	10886